(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
6 March 2003 (06.03.2003)

PCT

(10) International Publication Number
WO 03/017940 A2

(51) International Patent Classification[7]: A61K

(21) International Application Number: PCT/US02/26924

(22) International Filing Date: 22 August 2002 (22.08.2002)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
09/944,493 22 August 2001 (22.08.2001) US

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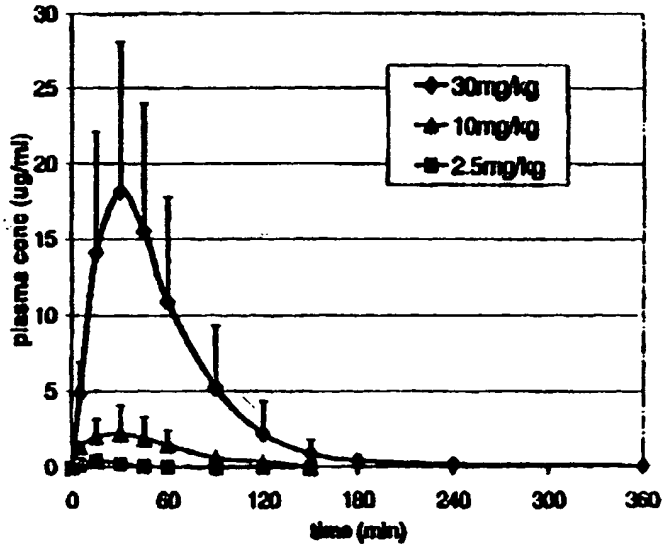
(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

[Continued on next page]

(54) Title: PULSATILE RELEASE COMPOSITIONS AND METHODS FOR ENHANCED GASTROINTESTINAL DRUG ABSORPTION



(57) Abstract: Modified release pharmaceutical formulations and methods for enhanced mucosal drug absorption. The formulation comprises initial population(s) of particles comprising both drug and penetration enhancer which are released at a first location in the gastrointestinal tract, and a subsequent population or populations of particles comprising a penetration enhancer(s) having a delayed release due to a polymeric coating or matrix. This penetration enhancer is released at an additional location(s) in the intestine downstream from the first location and enhances absorption of the drug when it reaches the additional location(s).

WO 03/017940 A2

Published:

— *without international search report and to be republished upon receipt of that report*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

PULSATILE RELEASE COMPOSITIONS AND METHODS FOR ENHANCED GASTROINTESTINAL DRUG ABSORPTION

FIELD OF THE INVENTION

[0001] The present invention relates to compositions and methods that enhance the gastrointestinal absorption of drugs, particularly oligonucleotides. More particularly, the invention relates to oral pharmaceutical formulations that deliver a first pulse of drug combined with a penetration enhancer and a second pulse of penetration enhancer to promote absorption of drug, which is not absorbed upon release with the first pulse of penetration enhancer.

BACKGROUND OF THE INVENTION

[0002] Advances in the field of biotechnology have led to significant advances in the treatment of diseases such as cancer, genetic diseases, arthritis and AIDS that were previouslydifficult to treat. Many such advances involve the administration of oligonucleotides and other nucleic acids to a subject, particularly a human subject. The administration of such molecules via parenteral routes has been shown to be effective for the treatment of diseases and/or disorders. See, e.g., Draper et al., U.S. Patent No. 5,595,978, January 21, 1997, which discloses intravitreal injection as a means for the direct delivery of antisense oligonucleotides to the vitreous humor of the mammalian eye. See also, Robertson, Nature Biotechnology, 1997, 15, 209, and Genetic Engineering News, 1997, 15, 1, each of which discuss the treatment of Crohn's disease via intravenous infusions of antisense oligonucleotides.

[0003] Oral administration of drugs, including oligonucleotides and other nucleic acids, offers the promise of simpler, easier and less injurious administration without the need for sterile procedures and their concomitant expenses, e.g., hospitalization and/or physician fees. However, the absorption of classes of drugs across mucosal barriers (i.e., oral, rectal, vaginal and nasal) is often poor.

[0004] Drugs may be classified into one of four categories based on their dose solubility and permeability properties. See, Amidon, et al., Pharm. Res. 12:413-420 (1995) which discusses this biopharmaceutic system to classify drugs based on their relevant physical and biophysical properties

that relate to their potential for absorption. For the most part both the pharmaceutical industry and regulatory communities acknowledge the validity of this system and expect most drug development efforts to consider these concepts. This is exemplified by the Food and Drug Administration's guidance issued in August 2000: "Waiver of In Vivo Bioavailability and Bioequivalence Studies for Immediate-Release Solid Oral Dosage Forms Based on a Biopharmaceutics Classification System." Briefly, drugs that exhibit either low (dose relevant) solubility, poor permeability, or both of these are prone to low absorption across mucosal barriers, such as the gastrointestinal epithelium in the case of orally administered drugs. Numerous effective methods have been devised to enhance the solubilization of poorly soluble drugs but, as yet, there is currently no effective and practical (i.e., noninvasive) means to deliver the majority of drugs that have poor permeability – particularly macromolecules such as oligonucleotides or proteins.

[0005] One approach to enhancing the absorption of orally administered drugs is pulsatile release formulations in which multiple doses of drug are released from a single formulation by the use of delayed release coatings (U.S. Patent Nos. 5,508,040, 6,117,450, 5,840,329, 5,814,336, and 5,686,105, the entire contents of which are incorporated herein by reference). There is a need to provide compositions and methods to enhance the absorption and bioavailability of orally administered drugs, particularly oligonucleotides.

SUMMARY OF THE INVENTION

[0006] Because of the advantages of oral delivery of drugs, including antisense oligonucleotides, the compositions and methods of the invention can be used in therapeutic methods as explained in more detail herein. The compositions and methods herein provided may also be used to examine the function of various proteins and genes in an animal, including those that are essential to animal development. The methods of the invention can be used, for example, for the treatments of animals that are known or suspected to suffer from any disease treatable with an oral pharmaceutically active compound, such as ulcerative colitis, rheumatoid arthritis, Crohn's disease, inflammatory bowel disease, or undue cellular proliferation.

[0007] One embodiment of the present invention is a delayed release oral formulation for enhanced intestinal drug absorption, comprising:

[0008] (a) a first population of particles comprising said drug and a penetration enhancer, wherein said drug and said penetration enhancer are released at a first location in the gastrointestinal tract; and

[0009] (b) one or more additional populations of particles comprising a penetration enhancer and a delayed release coating or matrix, wherein the penetration enhancer is released at one or more additional locations in the gastrointestinal tract downstream from the first location, whereby absorption of the drug is enhanced when the drug reaches the additional location or locations.

[0010] Preferably, the drug is a large macromolecule such as a protein, peptide, nucleic acid (DNA or RNA), oligonucleotide, enzyme, metabolism controlling agent, protease inhibitor, chemotherapeutic agent, angiotensin converting enzyme (ACE) inhibitor, vaccine, monoclonal antibody or polyclonal antibody. In one aspect of this preferred embodiment, the oligonucleotide is an antisense oligonucleotide. Preferably, the penetration enhancer in (a) and (b) is the same. Alternatively, the penetration enhancer in (a) and (b) is different. In one aspect of this preferred embodiment, the penetration enhancer is a fatty acid, bile acid, chelating agent, anionic, cationic or nonionic surfactant or non-chelating non-surfactant, or pharmaceutically acceptable salt thereof.

[0011] Advantageously, the fatty acid is arachidonic acid, oleic acid, lauric acid, capric acid, caprylic acid, myristic acid, palmitic acid, stearic acid, linoleic acid, linolenic acid, dicaprate, tricaprate, monoolein, dilaurin, glyceryl 1-monocaprate, 1-dodecylazacycloheptan-2-one, an acylcarnitine, an acylcholine, a monoglyceride or a pharmaceutically acceptable salt thereof. Fatty acids include those with 8-20 carbon atoms, either saturated or having one or more unsaturated bonds, and salts and glycerides thereof.

[0012] Preferably, the bile acid is cholic acid, dehydrocholic acid, deoxycholic acid, glucholic acid, glycholic acid, glycodeoxycholic acid, taurocholic acid, taurodeoxycholic acid, chenodeoxycholic acid, ursodeoxycholic acid, sodium tauro-24,25-dihydrofusidate, sodium glycodihydrofusidate, or a pharmaceutically acceptable salt thereof. In one aspect of this preferred embodiment, the chelating agent is EDTA, EGTA, citric acid, a salicylate, an N-acyl derivative of collagen, an N-amino acyl derivative of a beta-diketone or a mixture thereof.

[0013] Surfactants include anionic, cationic and non-ionic surfactants such as ethylene and/or propylene oxide derivatives, polyoxyethylene alkyl ethers and esters, polysorbates, poloxamers, sodium alkyl sulfates and polyethylene glycol derivatives

[0014] Advantageously, the non-chelating non-surfactant is an unsaturated cyclic urea, 1-alkyl-alkanone, 1-alkenylazacycloalkanone, steroid anti-inflammatory agent or mixtures thereof. Preferably, the formulation is a capsule, tablet, compression coated tablet, bilayer tablet, trilayer tablet, sachet, liquid-filled capsule or capsule comprising both liquid and solid components. In one aspect of this preferred embodiment, bioadhesive carrier particles are utilized. Advantageously, the carrier particles comprise poly-amino acids, polyimines, polyacrylates, polyalkylacrylates, polyoxethanes, polyalkylcyanoacrylates, cationized gelatins, albumins, starches, acrylates, polyethylene glycol, DEAE-derivatized polyimines, pollulans, celluloses, chitosan, poly-L-lysine, polyhistidine, polyornithine, polyspermines, protamine, polyvinylpyridine, polythiodiethylamino-methylene P(TDAE), polyaminostyrene, poly(methylcyanoacrylate), poly(ethylcyanoacrylate), poly(butylcyanoacrylate), poly(isobutylcyanoacrylate), poly(isohexylcyanoacrylate), DEAE-methacrylate, DEAE-ethyhexylacrylate, DEAE-acrylamide, DEAE-albumin, DEAE-dextran, polymethylacrylate, polyhexylacrylate, poly (D,L-lactic acid), poly (DL-lactic-coglycolic acid) (PLGA) or polyethylene glycol (PEG). In one aspect of this preferred embodiment, the carrier particles are cationic. Advantageously, the carrier particles comprise a complex of poly-L-lysine and alginate, a complex of protamine and alginate, lysine, dilysine, trilysine, calcium, albumin, glucosamine, arginine, galactosamine, nicotinamide, creatine, lysine-ethyl ester or arginine ethyl-ester. Preferably, the delayed release coating or matrix is acetate phthalate, propylene glycol, sorbitan monoleate, cellulose acetate phthalate (CAP), cellulose acetate trimellitate, hydroxypropyl methyl cellulose phthalate (HPMCP), methacrylates, chitosan, guar gum, polyethylene glycol (PEG), hydroxypropylmethylcellulose (HPMC), hydroxypropylethylcellulose, ethylcellulose or hydroxypropylmethylcellulose acetate succinate (HPMC-AS).

[0015] Preferably, all populations of particles comprise approximately equal portions (i.e, about 50%) of the penetration enhancer. More preferably, each population of particles comprises a different proportion of the penetration enhancer. the first population of carrier particles comprises about 50% of the penetration enhancer and the second population of carrier particles comprises about 50% of the penetration enhancer. More preferably, the first population of carrier particles comprises about 70% of thepenetration enhancer and said second population of carrier particles comprises about 30% of the penetration enhancer

DETAILED DESCRIPTION OF THE DRAWINGS

[0016] Figure 1 is a graph showing the plasma concentration of oligonucleotide over time after intrajejunal administration to monkeys. Saturation of oligonucleotide uptake pathways does not occur at the doses studied.

[0017] Figure 2 is a graph showing the plasma concentration of oligonucleotide in monkeys after a 30 minute infusion into a region of intestine made permeable by a 50 mg/kg bolus of sodium caprate at t=5 minutes.

[0018] Figures 3A-B are schematic diagrams showing the concentration of penetration enhancer in the intestinal lumen after administration of an immediate release formulation (Fig. 3A) and the pulsatile release formulation of the present invention (Fig. 3B).

[0019] Figure 4 is a graph showing the plasma bioavalability of the antisense oligonucleotide ISIS 104838 after oral administration to healthy human volunteers. IR=immediate release; 50/50 means that 50% of the sodium caprate, together with 100% of the oligonucleotide, is in the uncoated IR minitablets and the other 50% is in the coated delayed release minitabs; 70/30 means that 70% of the sodium caprate, together with 100% of the oligonuicleotide, is in the uncoated IR minitablets and the other 30% is in the coated delayed release minitabs. Fast comprises a lower level of delayed relese coating on the second pulse minitablets than the amount of coating on the second pulse for slow.

DETAILED DESCRIPTION OF THE INVENTION

[0020] The present invention provides oral pharmaceutical compositions that result in enhanced gastrointestinal absorption of biologically active substances. In particular, the present invention provides compositions and methods for enhancing the gastrointestinal absorption of drugs, preferably antisense oligonucleotides and other nucleic acids, thereby circumventing the complications and expense which may be associated with intravenous and other parenteral routes of administration. This enhancement is obtained by encapsulating at least two populations of particles. The first population of particles comprises a biologically active substance and a penetration enhancer, and the second (and optionally additional) population of particles comprises a penetration enhancer and a delayed release coating or matrix.

[0021] It is also contemplated that these pharmacutical compositons are capable of enhancing absorption of biologically active substances when administered via the rectal, vaginal, nasal or pulmonary routes. It is also contemplated that release of the biologically active substance can be achieved in any part of the gastrointestinal tract.

[0022] Enhanced bioavailability of biologically active substances is also achieved via the oral administration of the compositions and methods of the present invention. The term "bioavailability" refers to a measurement of what portion of an administered drug reaches the circulatorysystem when a non-parenteral mode of administration is used to introduce the drug into an animal. The term is used for drugs whose efficacy is related to the blood concentration achieved, even if the drug's ultimate site of action is intracellular (van Berge-Henegouwen et al., Gastroenterol., 1977, 73, 300). Traditionally, bioavailability studies determine the degree of intestinal absorption of a drug by measuring the change in peripheral blood levels of the drug after an oral dose (DiSanto, Chapter 76 In: Remington's Pharmaceutical Sciences, 18th Ed., Gennaro, ed., Mack Publishing Co., Easton, PA, 1990, pages 1451-1458). The area under the curve (AUC0) is divided by the area under the curve after an intravenous (i.v.) dose (AUCiv) and the quotient is used to calculate the fraction of drug absorbed. This approach cannot be used, however, with compounds which have a large "first pass clearance," i.e., compounds for which hepatic uptake is so rapid that only a fraction of the absorbed material enters the peripheral blood. For such compounds, other methods must be used to determine the absolute bioavailability (van Berge-Henegouwen et al., Gastroenterol., 1977, 73, 300). With regards to oligonucleotides, studies suggest that they are rapidly eliminated from plasma and accumulate mainly in the liver and kidney after i.v. administration (Miyao et al., Antisense Res. Dev., 1995, 5, 115; Takakura et al., Antisense & Nucl. Acid Drug Dev., 1996, 6, 177

[0023] Another "first pass effect" that applies to orally administereddrugs is degradation due to the action of gastric acid and various digestive enzymes. Furthermore, the entry of many high molecular weight active agents (such as peptides, proteins and oligonucleotides) and some conventional and/or low molecular weight drugs (e.g., insulin, vasopressin, leucine enkephalin, etc.) through mucosal routes (such as oral, pulmonary, buccal, rectal, transdermal, vaginal and ocular) to the bloodstream is frequently obstructed by poor transport across epithelial cells and concurrent metabolism during transport. This type of degradative metabolism is known for oligonucleotides and nucleic acids. For example, phosphodiesterases are known to cleave the phosphodiester linkages

of oligonucleotides and many other modified linkages present in synthetic oligonucleotides and nucleic acids.

[0024] One means of ameliorating first pass clearance effects is to increase the dose of administered drug, thereby compensating for proportion of drug lost to first pass clearance. Although this may be readily achieved with i.v. administration by, for example, simply providing more of the drug to an animal, other factors influence the bioavailability of drugs administered via non-parenteral means. For example, a drug may be enzymatically or chemically degraded in the alimentary canal or blood stream and/or may be impermeable or semipermeable to various mucosal membranes.

[0025] The delayed release pulsatile pharmaceutical formulations of the present invention comprise at least two populations of particles. The first population of particles comprises a biologically active substance of interest and a penetration enhancer, also known as an absorption enhancer. These are substances which facilitate the transport of a biologically active substance across mucosal surfaces and other epithelial cell membranes, particularly the intestinal mucosa. The first population of particles is released from the formulation at a first location in the gastrointestinal tract (i.e., intestine) and quickly release the biologically active substance and the penetration enhancer (first pulse). The penetration enhancer promotes absorption of the biologically active substance; however, because the enhancer is quickly absorbed, there is often an insufficient amount of enhancer to promote absorption of the entire dose of biologically active substance. The present invention solves this problem by providing a second (and optionally additional) population of particles comprised of a penetration enhancer and a delayed release coating or matrix. Because of the delayed release coating or matrix, the penetration enhancer in the second population of particles is released in the gastrointestinal tract downstream from the first location where it promotes further absorption when the biologically active substance reaches this site (second pulse). The penetration enhancer in the first population of particles may be either the same or different from the penetration enhancer in the second set of particles. The percentage of penetration enhancer in the first population of particles may be between about 1%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, or 90% of the total amount of penetration enhancer in the dosage form. The percentage of penetration enhancer in the second population of particles is between about 99%, 95%, 90%, 80%, 70%, 60%, 50%, 40%, 30%, 20%, or 10% of the total amount of penetration enhancer in the dosage

form. In a preferred embodiment, the penetration enhancer is evenly divided between all particle populations in the dosage form. In a particularly preferred embodiment, the penetration enhancer is not divided evenly between the various populations of particles. Most preferably, the first population comprises at least 50% of the penetration ehnancer. The delay time can be adjusted by varying the thickness of the delayed release coating on the second population of particles which is known to one of ordinary skill in the art, such that a thicker coating results in a longer delay time. Alternatively, the delay time may be adjusted by varying the amount of delayed release matrix.

[0026] Biologically active substance refers to any molecule or mixture or complex of molecules that exerts a biological effect in vitro and/or in vivo, including pharmaceuticals, drugs, proteins, vitamins, steroids, polyanions, nucleosides, nucleotides, oligonucleotides, antibodies, polynucleotides, etc. Preferably, poorly absorbed macromolecules are used in the formulations of the present invention.

[0027] Drugs refer to any therapeutic or prophylatic agent which is used in the prevention, diagnosis, alleviation, treatment or cure of a disease in an animal, particularly a human. Therapeutically useful oligonucleotides and polypeptides are within the scope of this definition for drugs.

[0028] Penetration enhancers include, but are not limited to, members of molecular classes such as surfactants, fatty acids, bile acids, chelating agents, non-chelating non-surfactant molecules, and salts thereof. (Lee et al., Critical Reviews in Therapeutic Drug Carrier Systems, 1991, p. 92). Carriers are inert molecules that may be included in the compositions of the present invention to interfere with processes that lead to reduction in the levels of bioavailable drug.

[0029] In connection with the present invention, surfactants (or "surface-active agents") are chemical entities which, when dissolved in an aqueous solution, reduce the surface tension of the solution or the interfacial tension between the aqueous solution and another liquid, with the result that absorption of oligonucleotides through the alimentary mucosa and other epithelial membranes is enhanced. In addition to bile acids, fatty acids and their salts, surfactants include, for example, sodium lauryl sulfate, polyoxyethylene-9-lauryl ether and polyoxyethylene-20-cetyl ether (Lee et al., Critical Reviews in Therapeutic Drug Carrier Systems, 1991, page 92); and perfluorochemical emulsions, such as FC-43 (Takahashi et al., J. Pharm. Pharmacol., 1988, 40, 252).

[0030] Fatty acids and their derivatives which act as penetration enhancers and may be used in compositions of the present invention include C8-C20 saturated or unsaturated, linear, branched or cyclic compounds, for example, oleic acid, lauric acid, capric acid (n-decanoic acid) (C10), myristic acid, palmitic acid, stearic acid, linoleic acid, linolenic acid, dicaprate, tricaprate, monoolein (1-monooleoyl-rac-glycerol), dilaurin, caprylic acid, arachidonic acid, glyceryl 1-monocaprate, 1-dodecylazacycloheptan-2-one, acylcarnitines, acylcholines and mono- and di-glycerides thereof and/or physiologically acceptable salts thereof (i.e., oleate, laurate, caprate, myristate, palmitate, stearate, linoleate, etc.) (Lee et al., Critical Reviews in Therapeutic Drug Carrier Systems, 1991, page 92; Muranishi, Critical Reviews in Therapeutic Drug Carrier Systems, 1990, 7, 1; El-Hariri et al., J. Pharm. Pharmacol., 1992, 44, 651).

[0031] A variety of bile acids and salts also function as penetration enhancers to facilitate the uptake and bioavailability of drugs. The physiological roles of bile include the facilitation of dispersion and absorption of lipids and fat-soluble vitamins (Brunton, Chapter 38 In: Goodman & Gilman's The Pharmacological Basis of Therapeutics, 9th Ed., Hardman et al., eds., McGraw-Hill, New York, NY, 1996, pages 934-935). Various natural bile salts, and their synthetic derivatives, act as penetration enhancers. Thus, the term "bile salt" includes any of the naturally occurring components of bile as well as any of their synthetic derivatives. The bile salts of the invention include, for example, cholic acid (or its pharmaceutically acceptable sodium salt, sodium cholate), dehydrocholic acid (sodium dehydrocholate), deoxycholic acid (sodium deoxycholate), glucholic acid (sodium glucholate), glycholic acid (sodium glycocholate), glycodeoxycholic acid (sodium glycodeoxycholate), taurocholic acid (sodium taurocholate), taurodeoxycholic acid (sodium taurodeoxycholate), chenodeoxycholic acid (CDCA, sodium chenodeoxycholate), ursodeoxycholic acid (UDCA), sodium tauro-24,25-dihydro-fusidate (STDHF) and sodium glycodihydrofusidate (Lee et al., Critical Reviews in Therapeutic Drug Carrier Systems, 1991, page 92; Swinyard, Chapter 39 In: Remington's Pharmaceutical Sciences, 18th Ed., Gennaro, ed., Mack Publishing Co., Easton, PA, 1990, pages 782-783; Muranishi, Critical Reviews in Therapeutic Drug Carrier Systems, 1990, 7, 1; Yamamoto et al., J. Pharm. Exp. Ther., 1992, 263, 25; Yamashita et al., J. Pharm. Sci., 1990, 79, 579).

[0032] Chelating agents, as used in connection with the present invention, can be defined to be compounds that remove metallic ions from solution by forming complexes therewith, with the result

that absorption of oligonucleotides through the alimentary and other mucosa is enhanced. With regards to their use as penetration enhancers in the present invention, chelating agents have the added advantage of also serving as DNase inhibitors, as most characterized DNA nucleases require a divalent metal ion for catalysis and are thus inhibited by chelating agents (Jarrett, J. Chromatogr., 1993, 618, 315). Chelating agents of the invention include, but are not limited to, disodium ethylenediaminetetraacetate (EDTA), EGTA, citric acid, salicylates (e.g., sodium salicylate, 5-methoxysalicylate and homovanilate), N-acyl derivatives of collagen and N-amino acyl derivatives of beta-diketones (enamines)(Lee et al., Critical Reviews in Therapeutic Drug Carrier Systems, 1991, page 92; Muranishi, Critical Reviews in Therapeutic Drug Carrier Systems, 1990, 7, 1; Buur et al., J. Control Rel., 1990, 14, 43).

[0033] As used herein, non-chelating non-surfactant penetration enhancers may be defined as compounds that demonstrate insignificant activity as chelating agents or as surfactants but that nonetheless enhance absorption of oligonucleotides through the alimentary and other mucosal membranes (Muranishi, Critical Reviews in Therapeutic Drug Carrier Systems, 1990, 7, 1). This class of penetration enhancers includes, but is not limited to, unsaturated cyclic ureas, 1-alkyl- and 1-alkenylazacyclo-alkanone derivatives (Lee et al., Critical Reviews in Therapeutic Drug Carrier Systems, 1991, page 92); and non-steroidal anti-inflammatory agents such as diclofenac sodium, indomethacin and phenylbutazone (Yamashita et al., J. Pharm. Pharmacol., 1987, 39, 621).

[0034] Agents that enhance uptake of oligonucleotides at the cellular level may also be added to the pharmaceutical and other compositions of the present invention. For example, cationic lipids, such as lipofectin (Junichi et al, U.S. Patent No. 5,705,188), cationic glycerol derivatives, and polycationic molecules, such as polylysine (Lollo et al., PCT Application WO 97/30731), can be used.

[0035] The pharmaceutical formulation into which the populations of particles are incorporated may be, for example, a capsule, tablet, compression coated tablet or bilayer tablet. In a preferred embodiment, these formulations comprise an enteric outer coating which resists degradation in the stomach and dissolves in the intestinal lumen. In a preferred embodiment, the formulation comprises an enteric material effective in protecting the nucleic acid from pH extremes of the stomach, or in releasing the nucleic acid over time to optimize the delivery thereof to a particular mucosal site. Enteric materials for acid-resistant tablets, capsules and caplets are known in the art

and typically include acetate phthalate, propylene glycol, sorbitan monoleate, cellulose acetate phthalate (CAP), cellulose acetate trimellitate, hydroxypropyl methyl cellulose phthalate (HPMCP), methacrylates, chitosan, guar gum, pectin, locust bean gum and polyethylene glycol (PEG). One particularly useful type of methacrylate are the EudragitsTM. These are anionic polymers that are water-impermeable at low pH, but become ionized and dissolve at intestinal pH. EUDRAGITSTM L100 and S100 are copolymers of methacrylic acid and methyl methacrylate.

[0036] Enteric materials may be incorporated within the dosage form or may be a coating substantially covering the entire surface of tablets, capsules or caplets. Enteric materials may also be accompanied by plasticizers that impart flexible resiliency to the material for resisting fracturing, for example during tablet curing or aging. Plasticizers are known in the art and typically include diethyl phthalate (DEP), triacetin, dibutyl sebacate (DBS), dibutyl phthalate (DBP) and triethyl citrate (TEC).

[0037] A "pharmaceutically acceptable" component of a formulation of the invention is one which, when used together with excipients, diluents, stabilizers, preservatives and other ingredients are appropriate to the nature, composition and mode of administration of a formulation. Accordingly it is desired to select penetration enhancers which facilitate the uptake of drugs, particularly oligonucleotides, without interfering with the activity of the drug and in a manner such that the same can be introduced into the body of an animal without unacceptable side-effects such as toxicity, irritation or allergic response.

[0038] A "particle" is defined herein as a granule, bead, microparticle, miniparticle, minitablet, nanoparticle or any other solid dosage form which can be incorporated into the oral pharmaceutical formulations described above.

A "carrier particle" is defined herein as a particle which comprises which primarily serves a function other than bioactive substance or penetration enhancer.

[0039] Preferred carrier particle-forming substances include poly-amino acids, polyimines, polyacrylates, dendrimers, polyalkylcyanoacrylates, cationized gelatins, albumins, starches, acrylates, polyethyleneglycols (PEG), DEAE-derivatized polyimines, pollulans and celluloses.

[0040] In other preferred embodiments, the carrier particle-forming substance includes polycationic polymers such as chitosan, poly-L-lysine, polyhistidine, polyornithine, polyspermines, protamine, polyvinylpyridine, polythiodiethylamino-methylene P(TDAE), polyaminostyrene (e.g.

para-amino), poly(methylcyanoacrylate), poly (ethylcyanoacrylate), poly (butylcyanoacrylate), poly(isobutylcyanoacrylate), poly(isohexylcyanoacrylate), DEAE-methacrylate, DEAE-hexylacrylate, DEAE-acrylamide, DEAE-albumin and DEAE-dextran. In another preferred embodiment, the particle-forming substance is poly-L-lysine complexed with alginate.

[0041] In an alternative embodiment, carrier particle-forming substances are non-polycationic, i.e., carry an overall neutral or negative charge, such as polyacrylates, for example polyalkylacrylates (e.g., methyl, hexyl), polyoxethanes, poly(DL-lactic-co-glycolic acid) (PLGA) and polyethyleneglycol.

[0042] In another embodiment, the pharmaceutical formulations of the invention may further comprise a bioadhesive material that serves to adhere particles to mucosal membranes. Carrier particles may themselves be bioadhesive, as is the case with PLL-alginate carrier particles, or may be coated with a bioadhesive material. Such materials are well known in the formulation art, examples of which are described in PCT WO85/02092, the contents of which are incorporated herein by reference. Preferred bioadhesive materials include polyacrylic polymers (e.g. carbomer and derivatives of carbomer), tragacanth, polyethyleneoxide cellulose derivatives (e.g. methylcellulose, carboxymethylcellulose, hydroxypropylmethylcellulose (HPMC), hydroxyethylcellulose (HEC), hydroxypropylcellulose (HPC) and sodium carboxymethylcellulose (NaCMC)), karya gum, starch, gelatin and pectin.

[0043] The formulations of the invention may further comprise a mucolytic substance which serves to degrade or erode mucin, partially or completely, at the site of the mucosal membrane to be traversed. Mucolytic substances are well known in the formulation art and include N-acetylcysteine, dithiothreitol, pepsin, pilocarpine, guaifenesin, glycerol guaiacolate, terpin hydrate, ammonium chloride, guattenesin, ambroxol, bromhexine, carbocysteine, domiodol, letosteine, mecysteine, mesna, sobrerol, stepronin, tiopronin and tyloxapol.

[0044] The drug may be associated with the carrier particles by electrostatic (e.g., ionic, polar, Van der Waals), covalent or mechanical (non-electrostatic, non-covalent) interactions depending on the drug and carrier particles, as well as the method of preparing the carrier particles. For example, an anionic drug such as an oligonucleotide can be bound to cationic carrier particles by ionic interaction.

[0045] The particles may also comprise an excipient. Typical pharmaceutical excipients include, but are not limited to, binding agents (e.g., pregelatinized maize starch, polyvinylpyrrolidone or hydroxypropyl methylcellulose, etc.); fillers (e.g., lactose and other sugars, microcrystalline cellulose, pectin, gelatin, calcium sulfate, ethyl cellulose, polyacrylates or calcium hydrogen phosphate, etc.); lubricants (e.g., magnesium stearate, talc, silica, colloidal silicon dioxide, stearic acid, metallic stearates, hydrogenated vegetable oils, corn starch, polyethylene glycols, sodium benzoate, sodium acetate, etc.); disintegrants (e.g., starch, sodium starch glycolate, EXPLOTAB); and wetting agents (e.g., sodium lauryl sulphate, etc.).

[0046] In a preferred embodiment, the second or later population of particles (comprising the penetration enhancer) further comprise a delayed release coating or matrix to delay dissolution until reaching a location in the gastrointestinal tract downstream from where the drug and penetration enhancer are released from the first population of particles which do not comprise a delayed release coating or matrix. This delayed release coating or matrix may be different from, or have a different thickness than, the delayed release coating or matrix on the pharmaceutical formulation (e.g. capsule or tablet) described above which causes release of the penetration enhancer after the combination of drug and penetration enhancer is released from the first population of particles. In a preferred embodiment, the coating on the second population of particles is not pH independent. An HPMC subcoat may be applied prior to the delayed release coating to provide an improved surface for adhesion of the delayed release coating.

[0047] There are three practical mechanisms by which a pharmaceutical formulation can be targeted into the intestine (small intestine or colon) following oral administration: activation by colonic bacterial enzymes or reducing environment created by the microflora, pH-dependent coating and time-dependent coating (coating thickness).

[0048] Delayed release coatings, and the properties which influence their dissolution, are well known in the art and are described in, for example, Bauer et al., Coated Pharmaceutical Dosage Forms, Medpharm Scientific Publishers, CRC Press, New York, 1998 and by Watts et al., Drug Devel, Industr. Pharm. 23:893-913, 1997, the entire contents of which are incorporated herein by reference.

[0049] The compositions of the present invention may additionally comprise other adjunct components conventionally found in pharmaceutical compositions, at their art-established usage

levels. Thus, the compositions may contain additional, compatible, pharmaceutically-active materials such as, for example, antipruritics, astringents, local anesthetics or anti-inflammatory agents, or may contain additional materials useful in physically formulating various dosage forms of the composition of present invention, such as dyes, flavoring agents, preservatives, antioxidants, opacifiers, thickening agents and stabilizers. However, such materials, when added, do not unduly interfere with the biological activities of the components of the compositions of the present invention.

[0050] The pharmaceutical compositions of the invention are used to deliver large macromolecular drugs (i.e., those having a molecular weight of greater than or equal to about 800 daltons) including peptides, proteins, monoclonal antibodies and fragments thereof, nucleic acids (DNA and RNA), oligonucleotides and antisense oligonucleotides, protease inhibitors, vaccines, monoclonal antibodies, polyclonal antibodies, metabolism controlling agents, angiotensin converting enzyme (ACE) inhibitors, as well as small molecules..

[0051] In a preferred embodiment, the pharmaceutical formulations are used to deliver oligonucleotides for use in antisense modulation of the function of DNA or messenger RNA (mRNA) encoding a protein the modulation of which is desired, and ultimately to regulate the amount of such a protein. Hybridization of an antisense oligonucleotide with its mRNA target interferes with the normal role of mRNA and causes a modulation of its function in cells. The functions of mRNA to be interfered with include all vital functions such as translocation of the RNA to the site for protein translation, actual translation of protein from the RNA, splicing of the RNA to yield one or more mRNA species, turnover or degradation of the mRNA and possibly even independent catalytic activity which may be engaged in by the RNA. The overall effect of such interference with mRNA function is modulation of the expression of a protein, wherein "modulation" means either an increase (stimulation) or a decrease (inhibition) in the expression of the protein. In the context of the present invention, inhibition is the preferred form of modulation of gene expression.

[0052] In the context of the present invention, the term "oligonucleotide" refers to an oligomer or polymer of ribonucleic acid or deoxyribonucleic acid. This term includes oligonucleotides composed of naturally occurring nucleobases, sugars and covalent intersugar (backbone) linkages as well as modified oligonucleotides having non-naturally-occurring portions that function similarly.

Such modified or substituted oligonucleotides are often preferred over native forms because of desirable properties such as, for example, enhanced cellular uptake, enhanced binding to target and increased stability in the presence of nucleases.

[0053] Oligonucleotides of the present invention may be, but are not limited to, those nucleic acids bearing modified linkages, modified nucleobases, or modified sugars, and chimeric nucleic acids.

A number of bioequivalents of oligonucleotides and other nucleic acids may also be employed in accordance with the present invention. The invention therefore, also encompasses oligonucleotide and nucleic acid equivalents such as, but not limited to, prodrugs of oligonucleotides and nucleic acids, deletion derivatives, conjugates of oligonucleotides, aptamers, and ribozymes.

[0054] An oligonucleotide is a polymer of repeating units generically known as nucleotides. An unmodified (naturally occurring) nucleotide has three components: (1) a nitrogenous base linked by one of its nitrogen atoms to (2) a 5-carbon cyclic sugar and (3) a phosphate, esterified to carbon 5 of the sugar. When incorporated into an oligonucleotide chain, the phosphate of a first nucleotide is also esterified to carbon 3 of the sugar of a second, adjacent nucleotide. The "backbone" of an unmodified oligonucleotide consists of (2) and (3), that is, sugars linked together by phosphodiester linkages between the carbon 5 (5') position of the sugar of a first nucleotide and the carbon 3 (3') position of a second, adjacent nucleotide. A "nucleoside" is the combination of (1) a nucleobase and (2) a sugar in the absence of (3) a phosphate moiety (Kornberg, A., DNA Replication, W.H. Freeman & Co., San Francisco, 1980, pages 4-7). The backbone of an oligonucleotide positions a series of bases in a specific order; the written representation of this series of bases, which is conventionally written in 5' to 3' order, is known as a nucleotide sequence.

[0055] Oligonucleotides may comprise nucleotide sequences sufficient in identity and number to effect specific hybridization with a particular nucleic acid. Such oligonucleotides which specifically hybridize to a portion of the sense strand of a gene are commonly described as "antisense." In the context of the invention, "hybridization" means hydrogen bonding, which may be Watson-Crick, Hoogsteen or reversed Hoogsteen hydrogen bonding, between complementary nucleotides. For example, adenine and thymine are complementary nucleobases that pair through the formation of hydrogen bonds. "Complementary," as used herein, refers to the capacity for precise pairing between two nucleotides. For example, if a nucleotide at a certain position of an

oligonucleotide is capable of hydrogen bonding with a nucleotide at the same position of a DNA or RNA molecule, then the oligonucleotide and the DNA or RNA are considered to be complementary to each other at that position. The oligonucleotide and the DNA or RNA are complementary to each other when a sufficient number of corresponding positions in each molecule are occupied by nucleotides which can hydrogen bond with each other. Thus, "specifically hybridizable" and "complementary" are terms which are used to indicate a sufficient degree of complementarity or precise pairing such that stable and specific binding occurs between the oligonucleotide and the DNA or RNA target. It is understood in the art that an oligonucleotide need not be 100% complementary to its target DNA sequence to be specifically hybridizable. An oligonucleotide is specifically hybridizable when binding of the oligonucleotide to the target DNA or RNA molecule interferes with the normal function of the target DNA or RNA to cause a decrease or loss of function, and there is a sufficient degree of complementarity to avoid non-specific binding of the oligonucleotide to non-target sequences under conditions in which specific binding is desired, i.e., under physiological conditions in the case of in vivo assays or therapeutic treatment, or in the case of in vitro assays, under conditions in which the assays are performed.

[0056] Antisense oligonucleotides are commonly used as research reagents, diagnostic aids, and therapeutic agents. For example, antisense oligonucleotides, which are able to inhibit gene expression with exquisite specificity, are often used by those of ordinary skill to elucidate the function of particular genes, for example to distinguish between the functions of various members of a biological pathway. This specific inhibitory effect has, therefore, been harnessed by those skilled in the art for research uses. Antisense oligonucleotides have also been used as diagnostic aids based on their specific binding or hybridization to DNA or mRNA that are present in certain disease states and due to the high degree of sensitivity that hybridization based assays and amplified assays that utilize some of polymerase chain reaction afford. The specificity and sensitivity of oligonucleotides is also harnessed by those of skill in the art for therapeutic uses. For example, the following U.S. patents demonstrate palliative, therapeutic and other methods utilizing antisense oligonucleotides. U. S. Patent No. 5,135,917 provides antisense oligonucleotides that inhibit human interleukin-1 receptor expression. U.S. Patent No. 5,098,890 is directed to antisense oligonucleotides complementary to the c-myb oncogene and antisense oligonucleotide therapies for certain cancerous conditions. U.S. Patent No. 5,087,617 provides methods for treating cancer patients with antisense

oligonucleotides. U.S. Patent No. 5,166,195 provides oligonucleotide inhibitors of Human Immunodeficiency Virus (HIV). U.S. Patent No. 5,004,810 provides oligomers capable of hybridizing to herpes simplex virus Vmw65 mRNA and inhibiting replication. U.S. Patent No. 5,194,428 provides antisense oligonucleotides having antiviral activity against influenzavirus. U.S. Patent No. 4,806,463 provides antisense oligonucleotides and methods using them to inhibit HTLV-III replication. U.S. Patent No. 5,286,717 provides oligonucleotides having a complementary base sequence to a portion of an oncogene. U.S. Patent No. 5,276,019 and U.S. Patent No. 5,264,423 are directed to phosphorothioate oligonucleotide analogs used to prevent replication of foreign nucleic acids in cells. U.S. Patent No. 4,689,320 is directed to antisense oligonucleotides as antiviral agents specific to cytomegalovirus (CMV). U.S. Patent No. 5,098,890 provides oligonucleotides complementary to at least a portion of the mRNA transcript of the human c-myb gene. U.S. Patent No. 5,242,906 provides antisense oligonucleotides useful in the treatment of latent Epstein-Barr virus (EBV) infections. Other examples of antisense oligonucleotides are provided herein.

[0057] Further, oligonucleotides used in the compositions of the present invention may be directed to modify the effects of mRNAs or DNAs involved in the synthesis of proteins that regulate adhesion of white blood cells and to other cell types. The adherence of white blood cells to vascular endothelium appears to be mediated in part if not in toto by five cell adhesion molecules ICAM-1, ICAM-2, ELAM-1, VCAM-1 and GMP-140. Dustin and Springer, J. Cell. Biol. 1987, 107, 321. Such antisense oligonucleotides are designed to hybridize either directly to the mRNA or to a selected DNA portion encoding intercellular adhesion molecule-1 (ICAM-1), endothelial leukocyte adhesion molecule-1 (ELAM-1, or E-selectin), and vascular cell adhesion molecule-1 (VCAM-1) as disclosed in U.S. Patents 5,514,788 (Bennett et al., May 7, 1996) and 5,591,623 (Bennett et al., January 7, 1997), and pending U.S. patent applications Serial Nos. 08/440,740 (filed May 12, 1995) and 09/062,416 (filed April 17, 1998). These oligonucleotides have been found to modulate the activity of the targeted mRNA, leading to the modulation of the synthesis and metabolism of specific cell adhesion molecules, and thereby result in palliative and therapeutic effects. Inhibition of ICAM-1, VCAM-1 and/or ELAM-1 expression is expected to be useful for the treatment of inflammatory diseases, diseases with an inflammatory component, allograft rejection, psoriasis and other skin diseases, inflammatory bowel disease, cancers and theirmetastases, and viral infection. Methods of

modulating cell adhesion comprising contacting the animal with an oligonucleotide composition of the present invention are provided.

[0058] Exemplary antisense compounds include the following:

ISIS 2302 is a 2'-deoxyoligonucleotide having a phosphorothioate backbone and the sequence 5'-GCC-CAA-GCT-GGC-ATC-CGT-CA-3' (SEQ ID NO:1). ISIS 2302 is targeted to the 3'-untranslated region (3'-UTR) of the human ICAM-1 gene. ISIS 2302 is described in U.S. Patents 5,514,788 and 5,591,623, hereby incorporated by reference.

[0059] ISIS 15839 is a phosphorothioate isosequence "hemimer" derivative of ISIS 2302 having the structure 5'-GCC-CAA-GCT-GGC-ATC-CGT-CA-3' (SEQ ID NO:1), wherein emboldened "C" residues have 5-methylcytosine (m5c) bases and wherein the emboldened, double-underlined residues further comprise a 2'-methoxyethoxy modification (other residues are 2'-deoxy). ISIS 15839 is described in co-pending U.S. Patent application Serial No. 09/062,416, filed April 17, 1998, hereby incorporated by reference.

[0060] ISIS 1939 is a 2'-oligodeoxynucleotide having a phosphorothioate backbone and the sequence 5'-CCC-CCA-CCA-CTT-CCC-CTC-TC-3' (SEQ ID NO:2). ISIS 1939 is targeted to the 3'-untranslated region (3'-UTR) of the human ICAM-1 gene. ISIS 1939 is described in U.S. Patents 5,514,788 and 5,591,623, hereby incorporated by reference.

[0061] ISIS 2302 (SEQ ID NO: 1) has been found to inhibit ICAM-1 expression in human umbilical vein cells, human lung carcinoma cells (A549), human epidermal carcinomacells (A431), and human keratinocytes. ISIS 2302 has also demonstrated specificity for its target ICAM-1 over other potential nucleic acid targets such as HLA-A and HLA-B. ISIS 1939 (SEQ ID NO:2) and ISIS 2302 markedly reduced ICAM-1 expression, as detected by northern blot analysis to determine mRNA levels, in C8161 human melanoma cells. In an experimental metastasis assay, ISIS 2302 decreased the metastatic potential of C8161 cells, and eliminated the enhanced metastatic ability of C8161 cells resulting from TNF-α treatment. ISIS 2302 has also shown significant biological activity in animal models of inflammatory disease. The data from animal testing has revealed strong anti-inflammatory effects of ISIS 2302 in a number of inflammatory diseases including Crohn's disease, rheumatoid arthritis, psoriasis, ulcerative colitis, and kidney transplant rejection. When tested on humans, ISIS 2302 has shown good safety and activity against Crohn's disease. Further ISIS 2302 has demonstrated a statistically significant steroid-sparing effect on treated subjects such

that even after five months post-treatment subjects have remained weaned from steroids and in disease remission. This is a surprising and significant finding of ISIS 2302's effects.

[0062] The oligonucleotides used in the compositions of the present invention preferably comprise from about 8 to about 30 nucleotides. It is more preferred that such oligonucleotides comprise from about 10 to about 25 nucleotides.

[0063] Antisense oligonucleotides employed in the compositions of the present invention may also be used to determine the nature, function and potential relationship of various genetic components of the body to normal or abnormal body states of animals. Heretofore, the function of a gene has been chiefly examined by the construction of loss-of-function mutations in the gene (i.e., "knock-out" mutations) in an animal (e.g., a transgenic mouse). Such tasks are difficult, time-consuming and cannot be accomplished for genes essential to animal development since the "knock-out" mutation would produce a lethal phenotype. Moreover, the loss-of-function phenotype cannot be transiently introduced during a particular part of the animal's life cycle or disease state; the "knock-out" mutation is always present. The use of "Antisense knockouts," that is, the selective modulation of expression of a gene by antisense oligonucleotides, rather than by direct genetic manipulation, overcomes these limitations (see, for example, Albert et al., Trends in Pharmacological Sciences, 1994, 15, 250). In addition, some genes produce a variety of mRNA transcripts as a result of processes such as alternative splicing; a "knock-out" mutation typically removes all forms of mRNA transcripts produced from such genes and thus cannot be used to examine the biological role of a particular mRNA transcript. By providing compositions and methods for the simple oral delivery of drugs, including oligonucleotides and other nucleic acids, the present invention overcomes these and other shortcomings.

[0064] Specific examples of some preferred modified oligonucleotides envisioned for use in the compositions of the present invention include oligonucleotides containing modified backbones or non-natural intersugar linkages. As defined in this specification, oligonucleotides having modified backbones include those that retain a phosphorus atom in the backbone and those that have an atom (or group of atoms) other than a phosphorus atom in the backbone. For the purposes of this specification, and as sometimes referenced in the art, modified oligonucleotides that do not have a phosphorus atom in their intersugar backbone, including peptide nucleic acids (PNAs) are also be considered to be oligonucleotides.

[0065] Specific oligonucleotide chemical modifications are described in the following subsections. It is not necessary for all positions in a given compound to be uniformly modified, and in fact more than one of the following modifications may be incorporated in a single antisense compound or even in a single residue thereof, for example, at a single nucleoside within an oligonucleotide.

[0066] **A. Modified Linkages**: Preferred modified oligonucleotide backbones include, for example, phosphorothioates, chiral phosphorothioates, phosphorodithioates, phosphotriesters, aminoalkylphosphotriesters, methyl and other alkyl phosphonates including 3'-alkylene phosphonates and chiral phosphonates, phosphinates, phosphoramidates including 3'-amino phosphoramidate and aminoalkylphosphoramidates, thionophosphoramidates, thionoalkylphosphonates, thionoalklyphosphotriesters, and boranophosphates having normal 3'-5' linkages, 2'-5' linked analogs of these, and those having inverted polarity wherein the adjacent pairs of nucleoside units are linked 3'-5' to 5'-3' or 2'-5' to 5'-2'. Various salts, mixed salts and free acid forms are also included.

[0067] Representative United States Patents that teach the preparation of the above phosphorus atom containing linkages include, but are not limited to, U.S. Patents Nos. 3,687,808; 4,469,863; 4,476,301; 5,023,243; 5,177,196; 5,188,897; 5,264,423; 5,276,019; 5,278,302; 5,286,717; 5,321,131; 5,399,676; 5,405,939; 5,453,496; 5,455,233; 5,466,677; 5,476,925; 5,519,126; 5,536,821; 5,541,306; 5,550,111; 5,563,253; 5,571,799; 5,587,361; 5,625,050; and 5,697,248, certain of which are commonly owned with this application, and each of which is herein incorporated by reference.

[0068] Preferred modified oligonucleotide backbones that do not include a phosphorus atom therein (i.e., oligonucleosides) have backbones that are formed by short chain alkyl or cycloalkyl intersugar linkages, mixed heteroatom and alkyl or cycloalkyl intersugar linkages, or one or more short chain heteroatomic or heterocyclic intersugar linkages. These include those having morpholino linkages (formed in part from the sugar portion of a nucleoside); siloxane backbones; sulfide, sulfoxide and sulfone backbones; formacetyl and thioformacetyl backbones; methylene formacetyl and thioformacetyl backbones; alkene containing backbones; sulfamate backbones; methyleneimino and methylenehydrazino backbones; sulfonate and sulfonamide backbones; amide backbones; and others having mixed N, O, S and CH2 component parts.

[0069] Representative United States patents that teach the preparation of the above oligonucleosides include, but are not limited to, U.S. Patents Nos. 5,034,506; 5,166,315; 5,185,444; 5,214,134; 5,216,141; 5,235,033; 5,264,562; 5,264,564; 5,405,938; 5,434,257; 5,466,677; 5,470,967; 5,489,677; 5,541,307; 5,561,225; 5,596,086; 5,602,240; 5,610,289; 5,602,240; 5,608,046; 5,610,289; 5,618,704; 5,623,070; 5,663,312; 5,633,360; 5,677,437; and 5,677,439, certain of which are commonly owned with this application, and each of which is herein incorporated by reference.

[0070] In other preferred oligonucleotide mimetics, both the sugar and the intersugar linkage, i.e., the backbone, of the nucleotide units are replaced with novel groups. The base units are maintained for hybridization with an appropriate nucleic acid target compound. One such oligomeric compound, an oligonucleotide mimetic that has been shown to have excellent hybridization properties, is referred to as a peptide nucleic acid (PNA). In PNA compounds, the sugar-backbone of an oligonucleotide is replaced with an amide containing backbone, in particular an aminoethylglycine backbone. The nucleobases are retained and are bound directly or indirectly to aza nitrogen atoms of the amide portion of the backbone. Representative United States patents that teach the preparation of PNA compounds include, but are not limited to, U.S. Patents Nos. 5,539,082; 5,714,331; and 5,719,262, each of which is herein incorporated by reference. Further teaching of PNA compounds can be found in Nielsen et al., Science, 1991, 254, 1497.

[0071] Some preferred embodiments of the present invention may employ oligonucleotides with phosphorothioate backbones and oligonucleosides with heteroatom backbones, and in particular- CH2-NH-O-CH2-, -CH2-N(CH3)-O-CH2- [known as a methylene (methylimino) or MMI backbone], -CH2-O-N(CH3)-CH2-,- CH2-N(CH3)-N(CH3)-CH2- and -O-N(CH3)-CH2-CH2- [wherein the native phosphodiester backbone is represented as -O-P-O-CH2-] of the above referenced U.S. Patent 5,489,677, and the amide backbones of the above referenced U.S. Patent No. 5,602,240. Also preferred are oligonucleotides having morpholino backbone structures of the above referenced U.S. Patent No. 5,034,506.

[0072] **B. Modified Nucleobases**: The oligonucleotides employed in the compositions of the present invention may additionally or alternatively comprise nucleobase (often referred to in the art simply as "base") modifications or substitutions. As used herein, "unmodified" or "natural" nucleobases include the purine bases adenine (A) and guanine (G), and the pyrimidine bases thymine

(T), cytosine (C) and uracil (U). Modified nucleobases include other synthetic and natural nucleobases such as 5-methylcytosine (5-me-C), 5-hydroxymethyl cytosine, xanthine, hypoxanthine, 2-aminoadenine, 6-methyl and other alkyl derivatives of adenine and guanine, 2-propyl and other alkyl derivatives of adenine and guanine, 2-thiouracil, 2-thiothymine and 2-thiocytosine, 5-halouracil and cytosine, 5-propynyl uracil and cytosine, 6-azo uracil, cytosine and thymine, 5-uracil (pseudouracil), 4-thiouracil, 8-halo, 8-amino, 8-thiol, 8-thioalkyl, 8-hydroxyl and other 8-substituted adenines and guanines, 5-halo particularly 5-bromo, 5-trifluoromethyl and other 5-substituted uracils and cytosines, 7-methylguanine and 7-methyladenine, 8-azaguanine and 8-azaadenine, 7-deazaguanine and 7-deazaadenine and 3-deazaguanine and 3-deazaadenine. Further nucleobases include those disclosed in United States Patent No. 3,687,808, those disclosed in the Concise Encyclopedia Of Polymer Science And Engineering, pages 858-859, Kroschwitz, J.I., ed. John Wiley & Sons, 1990, those disclosed by Englisch et al., Angewandte Chemie, International Edition, 1991, 30, 613, and those disclosed by Sanghvi, Y.S., Chapter 15, Antisense Research and Applications, pages 289-302, Crooke, S.T. and Lebleu, B., ed., CRC Press, 1993. Certain of these nucleobases are particularly useful for increasing the binding affinity of the oligomeric compounds of the invention. These include 5-substituted pyrimidines, 6-azapyrimidines and N-2, N-6 and O-6 substituted purines, including 2-aminopropyladenine, 5-propynyluracil and 5-propynylcytosine. 5-methylcytosine substitutions have been shown to increase nucleic acid duplex stability by 0.6-1.2 °C (Id., pages 276-278) and are presently preferred base substitutions, even more particularly when combined with 2'-methoxyethyl sugar modifications.

[0073] Representative United States patents that teach the preparation of certain of the above noted modified nucleobases as well as other modified nucleobases include, but are not limited to, the above noted U.S. Patent 3,687,808, as well as U.S. Patents 4,845,205; 5,130,302; 5,134,066; 5,175,273; 5,367,066; 5,432,272; 5,457,187; 5,459,255; 5,484,908; 5,502,177; 5,525,711; 5,552,540; 5,587,469; 5,594,121, 5,596,091; 5,614,617; and 5,681,941, certain of which are commonly owned, and each of which is herein incorporated by reference, and commonly owned United States patent application 08/762,488, filed on December 10, 1996, also herein incorporated by reference.

[0074] **C. Sugar Modifications**: The oligonucleotides employed in the compositions of the present invention may additionally or alternatively comprise one or more substituted sugar moieties.

Preferred oligonucleotides comprise one of the following at the 2' position: OH; F; O-, S-, or N-alkyl, O-, S-, or N-alkenyl, or O, S- or N-alkynyl, wherein the alkyl, alkenyl and alkynyl may be substituted or unsubstituted C1 to C10 alkyl or C2 to C10 alkenyl and alkynyl. Particularly preferred are O[(CH2)nO]mCH3, O(CH2)nOCH3, O(CH2)nNH2, O(CH2)nCH3, O(CH2)nONH2, and O(CH2)nON[(CH2)nCH3)]2, where n and m are from 1 to about 10. Other preferred oligonucleotides comprise one of the following at the 2' position: C1 to C10 lower alkyl, substituted lower alkyl, alkaryl, aralkyl, O-alkaryl or O-aralkyl, SH, SCH3, OCN, Cl, Br, CN, CF3, OCF3, SOCH3, SO2CH3, ONO2, NO2, N3, NH2, heterocycloalkyl, heterocycloalkaryl, aminoalkylamino, polyalkylamino, substituted silyl, an RNA cleaving group, a reporter group, an intercalator, a group for improving the pharmacokinetic properties of an oligonucleotide, or a group for improving the pharmacodynamic properties of an oligonucleotide, and other substituents having similar properties. A preferred modification includes 2'-methoxyethoxy [2'-O-CH2CH2OCH3, also known as 2'-O-(2-methoxyethyl) or 2'-MOE] (Martin et al., Helv. Chim. Acta, 1995, 78, 486), i.e., an alkoxyalkoxy group. A further preferred modification includes 2'-dimethylaminooxyethoxy, i.e., a O(CH2)2ON(CH3)2 group, also known as 2'-DMAOE, as described in co-owned United States patent application Serial Number 09/016,520, filed on January 30, 1998, the contents of which are herein incorporated by reference.

[0075] Other preferred modifications include 2'-methoxy (2'-O-CH3), 2'-aminopropoxy (2'-OCH2CH2CH2NH2) and 2'-fluoro (2'-F). Similar modifications may also be made at other positions on the oligonucleotide, particularly the 3' position of the sugar on the 3' terminal nucleotide or in 2'-5' linked oligonucleotides and the 5' position of 5' terminal nucleotide. Oligonucleotides may also have sugar mimetics such as cyclobutyl moieties in place of the pentofuranosyl sugar. Representative United States patents that teach the preparation of such modified sugars structures include, but are not limited to, U.S. Patents Nos. 4,981,957; 5,118,800; 5,319,080; 5,359,044; 5,393,878; 5,446,137; 5,466,786; 5,514,785; 5,519,134; 5,567,811; 5,576,427; 5,591,722; 5,597,909; 5,610,300; 5,627,0531 5,639,873; 5,646,265; 5,658,873; 5,670,633; and 5,700,920, certain of which are commonly owned, and each of which is herein incorporated by reference, and commonly owned United States patent application 08/468,037, filed on June 5, 1995, also herein incorporated by reference.

[0076] **D. Other Modifications**: Additional modifications may also be made at other positions on the oligonucleotide, particularly the 3' position of the sugar on the 3' terminal nucleotide and the 5' position of 5' terminal nucleotide. For example, one additional modification of the oligonucleotides employed in the compositions of the present invention invdves chemically linking to the oligonucleotide one or more moieties or conjugates which enhance the activity, cellular distribution or cellular uptake of the oligonucleotide. Such moieties include but are not limited to lipid moieties such as a cholesterol moiety (Letsinger et al., Proc. Natl. Acad. Sci. USA, 1989, 86, 6553), cholic acid (Manoharan et al., Bioorg. Med. Chem. Lett., 1994, 4, 1053), a thioether, e.g., hexyl-S-tritylthiol (Manoharan et al., Ann. N.Y. Acad. Sci., 1992, 660, 306; Manoharan et d., Bioorg. Med. Chem. Let., 1993, 3, 2765), a thiocholesterol (Oberhauser et al., Nucl. Acids Res., 1992, 20, 533), an aliphatic chain, e.g., dodecandiol or undecyl residues (Saison-Behmoaras et al., EMBO J., 1991, 10, 111; Kabanov et al., FEBS Lett., 1990, 259, 327; Svinarchuk et al., Biochimie, 1993, 75, 49), a phospholipid, e.g., di-hexadecyl-rac-glycerol or triethylammonium 1,2-di-O-hexadecyl-rac-glycero-3-H-phosphonate (Manoharan et al., Tetrahedron Lett., 1995, 36, 3651; Shea et al., Nucl. Acids Res., 1990, 18, 3777), a polyamine or a polyethylene glycol chain (Manoharan et al., Nucleosides & Nucleotides, 1995, 14, 969), or adamantane acetic acid (Manoharan et al., Tetrahedron Lett., 1995, 36, 3651), a palmityl moiety (Mishra et al., Biochim. Biophys Acta, 1995, 1264, 229), or an octadecylamine or hexylamino-carbonyl-oxycholesterol moiety (Crooke et al., J. Pharmacol. Exp. Ther., 1996, 277, 923).

[0077] Representative United States patents that teach the preparation of such oligonucleotide conjugates include, but are not limited to, U.S. Patents Nos. 4,828,979; 4,948,882; 5,218,105; 5,525,465; 5,541,313; 5,545,730; 5,552,538; 5,578,717, 5,580,731; 5,580,731; 5,591,584; 5,109,124; 5,118,802; 5,138,045; 5,414,077; 5,486,603; 5,512,439; 5,578,718; 5,608,046; 4,587,044; 4,605,735; 4,667,025; 4,762,779; 4,789,737; 4,824,941; 4,835,263; 4,876,335; 4,904,582; 4,958,013; 5,082,830; 5,112,963; 5,214,136; 5,082,830; 5,112,963; 5,214,136; 5,245,022; 5,254,469; 5,258,506; 5,262,536; 5,272,250; 5,292,873; 5,317,098; 5,371,241, 5,391,723; 5,416,203, 5,451,463; 5,510,475; 5,512,667; 5,514,785; 5,565,552; 5,567,810; 5,574,142; 5,585,481; 5,587,371; 5,595,726; 5,597,696; 5,599,923; 5,599,928 and 5,688,941, certain of which are commonly owned, and each of which is herein incorporated by reference.

[0078] A preferred conjugate imparting improved absorption of oligonucleotides in the gut is folic acid. Accordingly, there is provided a composition for oral administration comprising an oligonucleotide and a carrier wherein said oligonucleotide is conjugated to folic acid. Folic acid (folate) may be conjugated to the 3' or 5' termini of oligonucleotides, to a nucleobase or to a 2' position of any of the sugar residues in the chain. Conjugation may be via any suitable chemical linker utilizing functional groups on the oligonucleotide and folate. Oligonucleotide-folate conjugates and methods in preparing are described in copending United States patent applications 09/098,166 (filed June 16, 1998) and 09/275,505 (filed March 24, 1999) both incorporated herein by reference.

[0079] **E. Chimeric Oligonucleotides**: The present invention also includes compositions employing antisense compounds which are chimeric compounds. "Chimeric" antisense compounds or "chimeras," in the context of this invention, are antisense compounds, particularly oligonucleotides, which contain two or more chemically distinct regions, each made up of at least one monomer unit, i.e., a nucleotide in the case of an oligonucleotide compound. These oligonucleotides typically contain at least one region wherein the oligonucleotide is modified so as to confer upon the oligonucleotide increased resistance to nuclease degradation, increased cellular uptake, and/or increased binding affinity for the target nucleic acid. An additional region of the oligonucleotide may serve as a substrate for enzymes capable of cleaving RNA:DNA or RNA:RNA hybrids. By way of example, RNase H is a cellular endonuclease which cleaves the RNA strand of an RNA:DNA duplex. Activation of RNase H, therefore, results in cleavage of the RNA target, thereby greatly enhancing the efficiency of oligonucleotide inhibition of gene expression. Consequently, comparable results can often be obtained with shorter oligonucleotides when chimeric oligonucleotides are used, compared to phosphorothioate oligodeoxynucleotides hybridizing to the same target region. Cleavage of the RNA target can be routinely detected by gel electrophoresis and, if necessary, associated nucleic acid hybridization techniques known in the art. RNase H-mediated target cleavage is distinct from the use of ribozymes to cleave nucleic acids.

[0080] For example, such "chimeras" may be "gapmers," i.e., oligonucleotides in which a central portion (the "gap") of the oligonucleotide serves as a substrate for, eg., RNase H, and the 5' and 3' portions (the "wings") are modified in such a fashion so as to have greater affinity for, or stability when duplexed with, the target RNA molecule but are unable to support nuclease activity

(e.g., 2'-fluoro- or 2'-methoxyethoxy- substituted). Other chimeras include "hemimers," that is, oligonucleotides in which the 5' portion of the oligonucleotide serves as a substrate for, e.g., RNase H, whereas the 3' portion is modified in such a fashion so as to have greater affinity for, or stability when duplexed with, the target RNA molecule but is unable to support nuclease activity (e.g., 2'-fluoro- or 2'-methoxyethoxy- substituted), or vice-versa.

[0081] A number of chemical modifications to oligonucleotides that confer greater oligonucleotide:RNA duplex stability have been described by Freier et al. (Nucl. Acids Res., 1997, 25, 4429). Such modifications are preferred for the RNase H-refractory portions of chimeric oligonucleotides and may generally be used to enhance the affinity of an antisense compound for a target RNA.

[0082] Chimeric antisense compounds of the invention may be formed as composite structures of two or more oligonucleotides, modified oligonucleotides, oligonucleosides and/or oligonucleotide mimetics as described above. Such compounds have also been referred to in the art as hybrids or gapmers. Representative United States patents that teach the preparation of such hybrid structures include, but are not limited to, U.S. Patents Nos. 5,013,830; 5,149,797; 5,220,007; 5,256,775; 5,366,878; 5,403,711; 5,491,133; 5,565,350; 5,623,065; 5,652,355; 5,652,356; and 5,700,922, certain of which are commonly owned, and each of which is herein incorporated by reference, and commonly owned and allowed United States patent application serial number 08/465,880, filed on June 6, 1995, also herein incorporated by reference.

[0083] The present invention also includes compositions employing oligonucleotides that are substantially chirally pure with regard to particular positions within the oligonucleotides. Examples of substantially chirally pure oligonucleotides include, but are not limited to, those having phosphorothioate linkages that are at least 75% Sp or Rp (Cook et al., U.S. Patent No. 5,587,361) and those having substantially chirally pure (Sp or Rp) alkylphosphonate, phosphoramidate or phosphotriester linkages (Cook, U.S. Patents Nos. 5,212,295 and 5,521,302).

[0084] The present invention further encompasses compositions employing ribozymes. Synthetic RNA molecules and derivatives thereof that catalyze highly specific endoribonuclease activities are known as ribozymes. (See, generally, U.S. Patent Nos. 5,543,508 and 5,545,729) The cleavage reactions are catalyzed by the RNA molecules themselves. In naturally occurring RNA molecules, the sites of self-catalyzed cleavage are located within highly conserved regions of RNA

secondary structure (Buzayan et al., Proc. Natl. Acad. Sci. U.S.A., 1986, 83, 8859; Forster et al., Cell, 1987, 50, 9). Naturally occurring autocatalytic RNA molecules have been modified to generate ribozymes which can be targeted to a particular cellular or pathogenic RNA molecule with a high degree of specificity. Thus, ribozymes serve the same general purpose as antisense oligonucleotides (i.e., modulation of expression of a specific gene) and, like oligonucleotides, are nucleic acids possessing significant portions of single-strandedness. That is, ribozymes have substantial chemical and functional identity with oligonucleotides and are thus considered to be equivalents for purposes of the present invention.

[0085] Other biologically active oligonucleotides may be formulated in the compositions of the invention and used for therapeutic, palliative or prophylactic purposes according to the methods of the invention. Such other biologically active oligonucleotides include, but are not limited to, antisense compounds including, inter alia, antisense oligonucleotides, antisense PNAs and ribozymes (described supra) and EGSs, as well as aptamers and molecular decoys (described infra).

[0086] Sequences that recruit RNase P are known as External Guide Sequences, hence the abbreviation "EGS." EGSs are antisense compounds that direct of an endogenous nuclease (RNase P) to a targeted nucleic acid (Forster et al., Science, 1990, 249, 783; Guerrier-Takada et al.,Proc. Natl. Acad. Sci. USA, 1997, 94, 8468).

[0087] Antisense compounds may alternatively or additionally comprise a synthetic moiety having nuclease activity covalently linked to an oligonucleotide having an antisense sequence instead of relying upon recruitment of an endogenous nuclease. Synthetic moieties having nuclease activity include, but are not limited to, enzymatic RNAs (as in ribozymes), lanthanide ion complexes, and the like (Haseloff et al., Nature, 1988, 334, 585; Baker et al., J. Am. Chem. Soc., 1997, 119, 8749).

[0088] Aptamers are single-stranded oligonucleotides that bind specific ligands via a mechanism other than Watson-Crick base pairing. Aptamers are typically targeted to, e.g., a protein and are not designed to bind to a nucleic acid (Ellington et al., Nature, 1990, 346, 818).

[0089] Molecular decoys are short double-stranded nucleic acids (including single-stranded nucleic acids designed to "fold back" on themselves) that mimic a site on a nucleic acid to which a factor, such as a protein, binds. Such decoys are expected to competitively inhibit the factor; that is, because the factor molecules are bound to an excess of the decoy, the concentration of factor bound

to the cellular site corresponding to the decoy decreases, with resulting therapeutic, palliative or prophylactic effects. Methods of identifying and constructing nucleic acid decoy molecules are described in, e.g., U.S. Patent No. 5,716,780.

Another type of bioactive oligonucleotide is an RNA-DNA hybrid molecule that can direct gene conversion of an endogenous nucleic acid (Cole-Strauss et al., Science, 1996, 273, 1386).

[0090] Examples of specific oligonucleotides and the target genes to which they inhibit, which may be employed in formulations of the present invention include:

ISIS-2302	GCCCA AGCTG GCATC CGTCA	(SEQ ID NO:1)	ICAM-1
ISIS-15839	GCCCA AGCTG GCATC CGTCA	(SEQ ID NO:1)	ICAM-1
ISIS-1939	CCCCC ACCAC TTCCC CTCTC	(SEQ ID NO:2)	ICAM-1
ISIS-2922	GCGTT TGCTC TTCTT CTTGC G	(SEQ ID NO:3)	HCMV
ISIS-13312	GCGTT TGCTC TTCTT CTTGC G	(SEQ ID NO:3)	HCMV
ISIS-3521	GTTCT CGCTG GTGAG TTTCA	(SEQ ID NO:4)	PKCα
ISIS-9605	GTTCT CGCTG GTGAG TTTCA	(SEQ ID NO:4)	PKCα
ISIS-9606	GTTCT CGCTG GTGAG TTTCA	(SEQ ID NO:4)	PKCα
ISIS-14859	AACTT GTGCT TGCTC	(SEQ ID NO:5)	PKCα
ISIS-2503	TCCGT CATCG CTCCT CAGGG	(SEQ ID NO:6)	Ha-ras
ISIS-5132	TCCCG CCTGT GACAT GCATT	(SEQ ID NO:7)	c-raf
ISIS-14803	GTGCT CATGG TGCAC GGTCT	(SEQ ID NO:8)	HCV
ISIS-28089	GTGTG CCAGA CACCC TATCT	(SEQ ID NO:9)	TNFα
ISIS-104838	GCTGA TTAGA GAGAG GTCCC	(SEQ ID NO:10)	TNFα
ISIS-2105	TTGCT TCCAT CTTCC TCGTC	(SEQ ID NO:11)	HPV

wherein (i) each oligo backbone linkage is a phosphorothioate linkage (except ISIS-9605) and (ii) each sugar is 2'-deoxy unless represented in bold font in which case it incorporates a 2'-O-methoxyethyl group and iii) underlined cytosine nucleosides incorporate a 5-methyl substituent on their nucleobase. ISIS-9605 incorporates natural phosphodiester bonds at the first five and last five linkages with the remainder being phosphorothioate linkages.

[0091] **F. Synthesis**: The oligonucleotides used in the compositions of the present invention may be conveniently and routinely made through the well-known technique of solid phase synthesis. Equipment for such synthesis is sold by several vendors including, for example, Applied Biosystems

(Foster City, CA). Any other means for such synthesis known in the art may additionally or alternatively be employed. It is also known to use similar techniques to prepare other oligonucleotides such as the phosphorothioates and alkylated derivatives.

[0092] **1. Synthesis of oligonucleotides**: Teachings regarding the synthesis of particular modified oligonucleotides may be found in the following U.S. patents or pending patent applications, each of which is commonly assigned with this application: U.S. PatentsNos. 5,138,045 and 5,218,105, drawn to polyamine conjugated oligonucleotides; U.S. Patent No. 5,212,295, drawn to monomers for the preparation of oligonucleotides having chiral phosphorus linkages; U.S. Patents Nos. 5,378,825 and 5,541,307, drawn to oligonucleotides having modified backbones; U.S. Patent No. 5,386,023, drawn to backbone modified oligonucleotides and the preparation thereof through reductive coupling; U.S. Patent No. 5,457,191, drawn to modified nucleobases based on the 3-deazapurine ring system and methods of synthesis thereof; U.S. Patent No. 5,459,255, drawn to modified nucleobases based on N-2 substituted purines; U.S. Patent No. 5,521,302, drawn to processes for preparing oligonucleotides having chiral phosphorus linkages; U.S. Patent No. 5,539,082, drawn to peptide nucleic acids; U.S. Patent No. 5,554,746, drawn to oligonucleotides having β-lactam backbones; U.S. Patent No. 5,571,902, drawn to methods and materials for the synthesis of oligonucleotides; U.S. Patent No. 5,578,718, drawn to nucleosides having alkylthio groups, wherein such groups may be used as linkers to other moieties attached at any of a variety of positions of the nucleoside; U.S. Patents Nos. 5,587,361 and 5,599,797, drawn to oligonucleotides having phosphorothioate linkages of high chiral purity; U.S. Patent No. 5,506,351, drawn to processes for the preparation of 2'-O-alkyl guanosine and related compounds, including 2,6-diaminopurine compounds; U.S. Patent No. 5,587,469, drawn to oligonucleotides having N-2 substituted purines; U.S. Patent No. 5,587,470, drawn to oligonucleotides having 3-deazapurines; U.S. Patents Nos. 5,223,168, issued June 29, 1993, and 5,608,046, both drawn to conjugated 4'-desmethyl nucleoside analogs; U.S. Patent Nos. 5,602,240, and 5,610,289, drawn to backbone modified oligonucleotide analogs; and U.S. patent application Serial No. 08/383,666, filed February 3, 1995, and U.S. Patent No. 5,459,255, drawn to, inter alia, methods of synthesizing 2'-fluoro-oligonucleotides.

[0093] **2. Bioequivalents**: The compositions of the present invention encompass any pharmaceutically acceptable compound that, upon administration to an animal including a human, is

capable of providing (directly or indirectly) the biologically active metabolite or residue thereof. Accordingly, for example, the disclosure is also drawn to "prodrugs" and "pharmaceutically acceptable salts" of the antisense compounds of the invention and other bioequivalents, including bioequivalents of penetration enhancers..

[0094] **A. Oligonucleotide Prodrugs**: The oligonucleotide and nucleic acid compounds employed in the compositions of the present invention may additionally or alternatively be prepared to be delivered in a "prodrug" form. The term "prodrug" indicates a therapeutic agent that is prepared in an inactive form that is converted to an active form (i.e., drug) within the body or cells thereof by the action of endogenous enzymes or other chemicals and/or conditions. In particular, prodrug versions of the antisense compounds may be prepared as SATE [(S-acetyl-2-thioethyl) phosphate] derivatives according to the methods disclosed in WO 93/24510 (Gosselin et al., published December 9, 1993).

[0095] **B. Pharmaceutically Acceptable Salts**: The term "pharmaceutically acceptable salts" refers to physiologically and pharmaceutically acceptable salts of the penetration enhancer, oligonucleotide and nucleic acid compounds employed in the compositions of the present invention (i.e., salts that retain the desired biological activity of the parent compound and do not impart undesired toxicological effects thereto), as well as pharmaceutically acceptable salts of penetration enhancers.

[0096] Pharmaceutically acceptable base addition salts are formed with metals or amines, such as alkali and alkaline earth metals or organic amines. Examples of metals used as cations are sodium, potassium, magnesium, calcium, ammonium, polyamines such as spermine and spermidine, and the like. Examples of suitable amines are chloroprocaine, choline, N,N'-dibenzylethylenediamine, diethanolamine, dicyclohexylamine, ethylenediamine, N-methylglucamine, and procaine (see, for example, Berge et al., "Pharmaceutical Salts," J. of Pharma Sci., 1977, 66:1). The base addition salts of said acidic compounds are prepared by contacting the free acid form with a sufficient amount of the desired base to produce the salt in the conventional manner. The free acid form may be regenerated by contacting the salt form with an acid and isolating the free acid in the conventional manner. The free acid forms differ fromtheir respective salt forms somewhat in certain physical properties such as solubility in polar solvents, but otherwise the salts are equivalent to their respective free acid for purposes of the present invention.

[0097] During the process of oligonucleotide synthesis, nucleoside monomers are attached to the chain one at a time in a repeated series of chemical reactions such as nucleoside monomer coupling, oxidation, capping and detritylation. The stepwise yield for each nucleoside addition is above 99%. That means that less than 1% of the sequence chain failed to be generated from the nucleoside monomer addition in each step as the total results of the incomplete coupling followed by the incomplete capping, detritylation and oxidation (Smith, *Anal. Chem.*, 1988, *60*, 381A). All the shorter oligonucleotides, ranging from (n-1), (n-2), *etc.*, to 1-mers (nucleotides), are present as impurities in the n-mer oligonucleotide product. Among the impurities, (n-2)-mer and shorter oligonucleotide impurities are present in very small amounts and can be easily removed by chromatographic purification (Warren *et al.*, Chapter 9 *In: Methods in Molecular Biology, Vol. 26: Protocols for Oligonucleotide Conjugates*, Agrawal, S., Ed., 1994, Humana Press Inc., Totowa, NJ, pages 233-264). However, due to the lack of chromatographic selectivity and product yield, some (n-1)-mer impurities are still present in the full-length (*i.e.*, n-mer) oligonucleotide product after the purification process. The (n-1) portion consists of the mixture of all possible single base deletion sequences relative to the n-mer parent oligonucleotide. Such (n-1) impurities can be classified as terminal deletion or internal deletion sequences, depending upon the position of the missing base (*i.e.*, either at the 5' or 3' terminus or internally). When an oligonucleotide containing single base deletion sequence impurities is used as a drug (Crooke, Hematologic Pathology, 1995, 9, 59), the terminal deletion sequence impurities will bind to the same target mRNA as the full length sequence but with a slightly lower affinity. Thus, to some extent, such impurities can be considered as part of the active drug component, and are thus considered to be bioequivalents for purposes of the present invention.

[0098] Pharmaceutically acceptable organic or inorganic carrier substances suitable for oral administration which do not deleteriously react with nucleic acids can also be used to formulate the compositions of the present invention. Suitable pharmaceutically acceptable carriers include, but are not limited to, water, salt solutions, alcohols, polyethylene glycols, gelatin, mannitol, lactose and other sugars and sugar derivatives, amylose, magnesium stearate, talc, silicic acid, viscous paraffin, colloidal silicon dioxide, hydroxymethylcellulose, polyvinylpyrrolidone and the like. The formulations can be sterilized and, if desired, mixed with auxiliary agents, e.g., lubricants, preservatives, flavorants, stabilizers, wetting agents, emulsifiers, salts for influencing osmotic

pressure, buffers, bulking agents, colorings flavorings and/or aromatic substances and the like which do not deleteriously interact with the nucleic acid(s) of the formulation.

[0099] The present invention provides compositions and methods for oral delivery of a drug to an animal. For purposes of the invention, the term "animal" is meant to encompass humans as well as other mammals, as well as reptiles, fish, amphibians, and birds.

[0100] Surfactants find wide application in formulations such as emulsions (including microemulsions) and liposomes. The most common way of classifying and ranking the properties of the many different types of surfactants, both natural and synthetic, is by the use of the hydrophile/lipophile balance (HLB). The nature of the hydrophilic group (also known as the "head") provides the most useful means for categorizing the different surfactants used in formulations (Rieger, in *Pharmaceutical Dosage Forms: Disperse Systems*, Vol. 1, Lieberman, Rieger and Banker, Eds., Marcel Dekker, Inc., New York, NY, 1988, p. 285).

[0101] If the surfactant molecule is not ionized, it is classified as a nonionic surfactant. Nonionic surfactants find wide application in pharmaceutical and cosmetic products and are usable over a wide range of pH values. In general their HLB values range from 2 to about 18 depending on their structure. Nonionic surfactants include nonionic ethers and esters such as ethylene glycol esters, propylene glycol ethers and esters, glyceryl esters, polyglyceryl eethers and esters, sorbitan esters, sucrose esters, and ethoxylated esters. Nonionic alkanolamides and ethers such as fatty alcohol ethoxylates, propoxylated alcohols, and ethoxylated/ propoxylated block polymers are also included in this class. The polyoxyethylene surfactants are the most popular members of the nonionic surfactant class.

[0102] If the surfactant molecule carries a negative charge when it is dissolved or dispersed in water, the surfactant is classified as anionic. Anionic surfactants include carboxylates such as soaps, acyl lactylates, acyl amides of amino acids, esters of sulfuric acid such as alkyl sulfates and ethoxylated alkyl sulfates, sulfonates such as alkyl benzene sulfonates, acyl isethionates, acyl taurates and sulfosuccinates, and phosphates. The most important members of the anionic surfactant class are the alkyl sulfates and the soaps.

[0103] If the surfactant molecule carries a positive charge when it is dissolved or dispersed in water, the surfactant is classified as cationic. Cationic surfactants include quaternary ammonium

salts and ethoxylated amines. The quaternary ammonium salts are the most used members of this class.

[0104] If the surfactant molecule has the ability to carry either a positive or negative charge, the surfactant is classified as amphoteric. Amphoteric surfactants include acrylic acid derivatives, substituted alkylamides, N-alkylbetaines and phosphatides.

[0105] The use of surfactants in drug products, formulations and in emulsions has been reviewed (Rieger, in *Pharmaceutical Dosage Forms: Disperse Systems*, Vol. 1, Lieberman, Rieger and Banker, Eds., Marcel Dekker, Inc., New York, NY, 1988, p. 285).

In a preferred embodiment of the invention, one or more nucleic acids are administered via mucosal delivery.

[0106] Compositions for mucosal administration include powders or granules, beads, suspensions or solutions in water or non-aqueous media, capsules, sachets, troches, tablets or SECs (soft elastic capsules or "caplets"). Thickeners, flavoring agents, colorants, emulsifiers, dispersing aids, carrier substances or binders may be desirably added to such formulations. A tablet may be made by compression or molding, optionally with one or more accessory ingredients.

[0107] Compressed tablets may be prepared by compressing in a suitable machine, the active ingredients in a free-flowing form such as a powder or granules, optionally mixed with a binder (PVP or gums such as tragacanth, acacia, carrageenan), lubricant (e.g. stearates such as magnesium stearate), glidant (talc, colloidal silica dioxide), inert diluent, preservative, surface active or dispersing agent. Preferred binders/disintegrants include EMDEX (dextrate), PRECIROL (triglyceride), PEG, and AVICEL (cellulose). Molded tablets may be made by molding in a suitable machine a mixture of the powdered compound moistened with an inert liquid diluent. The tablets may optionally be coated or scored and may be formulated so as to provide slow or controlled release of the active ingredients therein.

[0108] Various methods for producing formulations for alimentary delivery arewell known in the art. See, generally, Nairn, Chapter 83; Block, Chapter 87; Rudnic *et al.*, Chapter 89; Porter, Chapter 90; and Longer *et al.*, Chapter 91 *In: Remington's Pharmaceutical Sciences*, 18th Ed., Gennaro, ed., Mack Publishing Co., Easton, PA, 1990. The compositions of this invention can be converted in a known manner into the customary formulations, such as tablets, coated tablets, pills, granules, capsules, aerosols, syrups, emulsions, suspensions and solutions, using inert, non-toxic,

pharmaceutically suitable excipients or solvents. The therapeutically active compound is present in a concentration of about 0.5% to about 95% by weight of the total mixture, that is to say in amounts which are sufficient to achieve the stated dosage range. Compositions may be formulated in a conventional manner using additional pharmaceutically acceptable carriers or excipients as appropriate. Thus, the composition may be prepared by conventional means with carriers or excipients such as binding agents (*e.g.*, pregelatinized maize starch, polyvinylpyrrolidone or hydroxypropyl methylcellulose); fillers (*e.g.*, lactose, microcrystalline cellulose or calcium hydrogen phosphate); lubricants (*e.g.*, magnesium stearate, talc or silica); disintegrants (*e.g*, starch or sodium starch glycolate); or wetting agents (*e.g.*, sodium lauryl sulfate). Tablets may be coated by methods well known in the art. The preparations may also contain flavoring, coloring and/or sweetening agents as appropriate.

[0109] Capsules used for oral delivery may include formulations that are well known in the art. Further, multicompartment hard capsules with controlled release properties as described by Digenis *et al.*, U.S. Patent No. 5,672,359, and water permeable capsules with a multi-stage drug delivery system as described by Amidon *et al.*, U.S. Patent No. 5,674,530 may also be used to formulate the compositions of the present invention. Capsules may be filled with powders, granules, beads or other multiparticulates, semi-solids, liquids, tablets, solid compacts, emulsions or any combination of these or similar compositions.

[0110] The formulation of pharmaceutical compositions and their subsequent administration is believed to be within the skill of those in the art. Specific comments regarding the present invention are presented below.

[0111] In general, for therapeutic applications, a patient (*i.e.*, an animal, including a human) having or predisposed to a disease or disorder is administered one or more drugs, preferably nucleic acids, including oligonucleotides, in accordance with the invention in a pharmaceutically acceptable carrier in doses ranging from 0.01 ug to 100 g per kg of body weight depending on the age of the patient and the severity of the disorder or disease state being treated. Further, the treatment regimen may last for a period of time which will vary depending upon the nature of the particular disease or disorder, its severity and the overall condition of the patient, and may extend from once daily to once every 20 years. In the context of the invention, the term "treatment regimen" is meant to encompass therapeutic, palliative and prophylactic modalities. Following treatment, the patient is monitored for

changes in his/her condition and for alleviation of the symptoms of the disorder or disease state The dosage of the drug may either be increased if the patient does not respond significantly to current dosage levels, or the dose may be decreased if an alleviation of the symptoms of the disorder or disease state is observed, or if the disorder or disease state has been abated.

[0112] Dosing is dependent on severity and responsiveness of the disease state to be treated, with the course of treatment lasting from several days to several months, or until a cure is effected or a diminution of the disease state is achieved. Optimal dosing schedules can be calculated from measurements of drug accumulation in the body of the patient. Persons of ordinary skill can easily determine optimum dosages, dosing methodologies and repetition rates. Optimum dosages may vary depending on the relative potency of individual drugs, and can generally be estimated based on EC_{50} values found to be effective in *in vitro* and *in vivo* animal models. In general, dosage is from 0.01 μg to 100 g per kg of body weight, and may be given once or more daily, weekly, monthly or yearly, or even once every 2 to 20 years. An optimal dosing schedule is used to deliver a therapeutically effective amount of the drug being administered via a particular mode of administration.

[0113] The term "therapeutically effective amount," for the purposes of the invention, refers to the amount of drug-containing formulation that is effective to achieve an intended purpose without undesirable side effects (such as toxicity, irritation or allergic response). Although individual needs may vary, optimal ranges for effective amounts of formulations can be readily determined by one of ordinary skill in the art. Human doses can be extrapolated from animal studies (Katocs *et al.*, Chapter 27 *In: Remington's Pharmaceutical Sciences*, 18th Ed., Gennaro, ed., Mack Publishing Co., Easton, PA, 1990). Generally, the dosage required to provide an effective amount of a formulation, which can be adjusted by one skilled in the art, will vary depending on the age, health, physical condition, weight, type and extent of the disease or disorder of the recipient, frequency of treatment, the nature of concurrent therapy (if any) and the nature and scope of the desired effect(s) (Nies *et al.*, Chapter 3 *In: Goodman & Gilman's The Pharmacological Basis of Therapeutics*, 9th Ed., Hardman *et al.*, eds., McGraw-Hill, New York, NY, 1996).

[0114] Following successful treatment, it may be desirable to have the patient undergo maintenance therapy to prevent the recurrence of the disease state, wherein the nucleic acid is administered in maintenance doses, ranging from 0.01 ug to 100 g per kg of body weight, once or more daily, to once every 20 years. For example, in the case of in individual known or suspected of

being prone to an autoimmune or inflammatory condition, prophylactic effects may be achieved by administration of preventative doses, ranging from 0.01 ug to 100 g per kg of body weight, once or more daily, to once every 20 years. In like fashion, an individual may be made less susceptible to an inflammatory condition that is expected to occur as a result of some medical treatment, *e.g.*, graft versus host disease resulting from the transplantation of cells, tissue or an organ into the individual.

[0115] Formulations for mucosal administration may include sterile and non-sterile aqueous solutions or suspensions, non-aqueous solutions in common solvents such as alcohols, or solutions or suspensions in liquid or solid oil bases. The solutions may also contain buffers, diluents and other suitable additives. Suspensions may contain substances that increase the viscosity of the suspension including, for example, sodium carboxymethylcellulose, sorbitol and/or dextran. The suspension may also contain stabilizers.

[0116] The pharmaceutical formulations, which may conveniently be presented in unit dosage form, may be prepared according to conventional techniques well known in the pharmaceutical industry. Such techniques include the step of bringing into association the active ingredients with the pharmaceutical carrier(s) or excipient(s). In general the formulations are prepared by uniformly and intimately bringing into association the active ingredients with liquid carriers or finely divided solid carriers or both, and then, if necessary, shaping the product.

[0117] In a preferred embodiment, the invention is directed to oral administration of a nucleic acid, such as an oligonucleotide, having biological activity, to an animal. By "having biological activity," it is meant that the nucleic acid functions to modulate the expression of oneor more genes in an animal as reflected in either absolute function of the gene (such as ribozyme activity) or by production of proteins coded by such genes. In the context of this invention, "to modulate" means to either effect an increase (stimulate) or a decrease (inhibit) in the expression of a gene. Such modulation can be achieved by, for example, an antisense oligonucleotide by a variety of mechanisms known in the art, including but not limited to transcriptional arrest; effects on RNA processing (capping, polyadenylation and splicing) and transportation; enhancement or reduction of cellular degradation of the target nucleic acid; and translational arrest (Crooke *et al., Exp. Opin. Ther. Patents*, 1996, *6,* 1).

[0118] In an animal other than a human, the compositions and methods of the invention can be used to study the function of one or more genes in the animal. For example, antisense

oligonucleotides have been systemically administered to rats in order to study the role of the *N*-methyl-D-aspartate receptor in neuronal death, to mice in order to investigate the biological role of protein kinase C-a, and to rats in order to examine the role of the neuropeptide Y1 receptor in anxiety (Wahlestedt *et al.*, *Nature*, 1993, *363,* 260; Dean *et al.*, *Proc. Natl. Acad. Sci. U.S.A.*, 1994, *91,* 11762; and Wahlestedt *et al.*, *Science*, 1993, *259,* 528, respectively). In instances where complex families of related proteins are being investigated, "antisense knockouts" (*i.e.*, inhibition of a gene by systemic administration of antisense oligonucleotides) may represent the most accurate means for examining a specific member of the family (see, generally, Albert *et al.*, *Trends Pharmacol. Sci.*, 1994, *15,* 250).

[0119] As stated, the compositions and methods of the invention are useful therapeutically, *i.e.*, to provide therapeutic, palliative or prophylactic relief to an animal, including a human, having or suspected of having or of being susceptible to, a disease or disorder that is treatable in whole or in part with one or more nucleic acids. The term "disease or disorder" (1) includes any abnormal condition of an organism or part, especially as a consequence of infection, inherent weakness, environmental stress, that impairs normal physiological functioning; (2) excludes pregnancy *per se* but not autoimmune and other diseases associated with pregnancy; and (3) includes cancers and tumors. The term "having or suspected of having or of being susceptible to" indicates that the subject animal has been determined to be, or is suspected of being, at increased risk, relative to the general population of such animals, of developing a particular disease or disorder as herein defined. For example, a subject animal could have a personal and/or family medical history that includes frequent occurrences of a particular disease or disorder. As another example, a subject animal could have had such a susceptibility determined by genetic screening according to techniques known in the art (see, *e.g.*, U.S. Congress, Office of Technology Assessment, Chapter 5*In: Genetic Monitoring and Screening in the Workplace,* OTA-BA-455, U.S. Government Printing Office, Washington, D.C., 1990, pages 75-99). The term "a disease or disorder that is treatable in whole or in part with one or more nucleic acids" refers to a disease or disorder, as herein defined, (1) the management, modulation or treatment thereof, and/or (2) therapeutic, palliative and/or prophylactic relief therefrom, can be provided via the administration of more nucleic acids. In a preferred embodiment, such a disease or disorder is treatable in whole or in part with an antisense oligonucleotide.

EXAMPLES

[0120] The following examples illustrate the invention and are not intended to limit the same. Those skilled in the art will recognize, or be able to ascertain through routine experimentation, numerous equivalents to the specific substances and procedures described herein. Such equivalents are considered to be within the scope of the present invention.

Example 1: Preparation of Oligonucleotides

[0121] **A. General Synthetic Techniques:** Oligonucleotides were synthesized on an automated DNA synthesizer using standard phosphoramidite chemistry with oxidation using iodine. Beta-cyanoethyldiisopropyl phosphoramidites were purchased from Applied Biosystems (Foster City, CA). For phosphorothioate oligonucleotides, the standard oxidation bottle was replaced by a 0.2 M solution of 3*H*-1,2-benzodithiole-3-one-1,1-dioxide in acetonitrile for the stepwise thiation of the phosphite linkages.

[0122] The synthesis of 2'-O-methyl- (2'-methoxy-) phosphorothioate oligonucleotides is according to the procedures set forth above substituting 2'-O-methyl b-cyanoethyldiisopropyl phosphoramidites (Chemgenes, Needham, MA) for standard phosphoramidites and increasing the wait cycle after the pulse delivery of tetrazole and base to 360 seconds.

[0123] Similarly, 2'-O-propyl- (a.k.a 2'-propoxy-) phosphorothioate oligonucleotides are prepared by slight modifications of this procedure and essentially according to procedures disclosed in U.S. patent application Serial No. 08/383,666, filed February 3, 1995, which is assigned to the same assignee as the instant application and which is incorporated by reference herein.

[0124] The 2'-fluoro-phosphorothioate oligonucleotides of the invention are synthesized using 5'-dimethoxytrityl-3'-phosphoramidites and prepared as disclosed in U.S. patent application Serial No. 08/383,666, filed February 3, 1995, and U.S. Patent 5,459,255, which issued October 8, 1996, both of which are assigned to the same assignee as the instant application and which are incorporated by reference herein. The 2'-fluoro-oligonucleotides are prepared using phosphoramidite chemistry and a slight modification of the standard DNA synthesis protocol (*i.e*, deprotection was effected using methanolic ammonia at room temperature).

[0125] PNA antisense analogs are prepared essentially as described in U.S. Patents Nos. 5,539,082 and 5,539,083, both of which (1) issued July 23, 1996, (2) are assigned to the same assignee as the instant application and (3) are incorporated by reference herein.

[0126] Oligonucleotides comprising 2,6-diaminopurine are prepared using compounds described in U.S. Patent No. 5,506,351 which issued April 9, 1996, and which is assigned to the same assignee as the instant application and incorporated by reference herein, and materials and methods described by Gaffney *et al.* (*Tetrahedron*, 1984, *40,* 3), Chollet *et al.*, (*Nucl. Acids Res.*, 1988, *16,* 305) and Prosnyak *et al.* (*Genomics*, 1994, *21,* 490). Oligonucleotides comprising 2,6-diaminopurine can also be prepared by enzymatic means (Bailly *et al.*, *Proc. Natl. Acad. Sci. U.S.A.*, 1996, *93,* 13623).

2'-Methoxyethoxy oligonucleotides of the invention are synthesized essentially according to the methods of Martin *et al.* (*Helv. Chim. Acta*, 1995, *78*, 486).

[0127] **B. Oligonucleotide Purification:** After cleavage from the controlled pore glass (CPG) column (Applied Biosystems) and deblocking in concentrated ammonium hydroxide, at 55°C for 18 hours, the oligonucleotides were purified by precipitation 2x from 0.5 M NaCl with 2.5 volumes of ethanol followed by further purification by reverse phase high liquid pressure chromatography (HPLC). Analytical gel electrophoresis was accomplished in 20% acrylamide, 8 M urea and 45 mM Tris-borate buffer (pH 7).

[0128] **C. Oligonucleotide Labeling:** Antisense oligonucleotides were labeled in order to detect the presence of and/or measure the quantity thereof in samples taken during the course of the *in vivo* pharmacokinetic studies described herein. Although radiolabeling by tritium exchange is one preferred means of labeling antisense oligonucleotides for such *in vivo* studies, a variety of other means are available for incorporating a variety of radiological, chemical or enzymatic labels into oligonucleotides and other nucleic acids.

[0129] **1. Tritium Exchange:** Essentially, the procedure of Graham *et al.* (*Nucleic Acids Research*, 1993, *21,* 3737) was used to label oligonucleotides by tritium exchange. Specifically, about 24 mg of oligonucleotide was dissolved in a mixture of 200μL of sodium phosphate buffer (pH 7.8), 400 μL of 0.1 mM EDTA (pH 8.3) and 200 μL of deionized water. The pH of the resulting mixture was measured and adjusted to pH 7.8 using 0.095*N* NaOH. The mixture was lyophilized overnight in a 1.25 mL gasketed polypropylene vial. The oligonucleotide was dissolved in 8.25 μL

of β-mercaptoethanol, which acts as a free radical scavenger (Graham *et al.*, *Nucleic Acids Research*, 1993, *21*, 3737), and 400 µL of tritiated H_2O (5 Ci/gram). The tube was capped, placed in a 90 °C oil bath for 9 hours without stirring, and then briefly centrifuged to remove any condensate from the inside lid of the tube. (As an optional analytical step, two 10 µL aliquots (one for HPLC analysis, one for PAGE analysis) were removed from the reaction tube; each aliquot was added to a separate 1.5 mL standard microfuge tube containing 490 µL of 50 uM sodium phosphate buffer (pH 7.8).) The oligonucleotide mixture is then frozen in liquid nitrogen and transferred to a lyophilization apparatus wherein lyophilization was carried out under high vacuum, typically for 3 hours. The material was then resuspended in mL of double-distilled water and allowed to exchange for 1 hour at room temperature. After incubation, the mixture was again quick frozen and lyophilized overnight. (As an optional analytical step, about 1 mg of the oligonucleotide material is removed for HPLC analysis.) Three further lyophilizations were carried out, each with approximately 1 mL of double-distilled water, to ensure the removal of any residual, unincorporated tritium. The final resuspended oligonucleotide solution is transferred to a clean polypropylene vial and assayed. The tritium labeled oligonucleotide is stored at about –70 °C.

[0130] 2. Other Means of Labeling Nucleic Acids: As is well known in the art, a variety of means are available to label oligonucleotides and other nucleic acids and to separate unincorporated label from the labeled nucleic acid. For example, double-stranded nucleic acids can be radiolabeled by nick translation and primer extension, and a variety of nucleic acids, including oligonucleotides, can be terminally radiolabeled by the use of enzymes such as T4 polynucleotide kinase or terminal deoxynucleotidyl transferase (see, generally, Chapter 3 *In: Short Protocols in Molecular Biology*, 2d Ed., Ausubel *et al.*, eds., John Wiley & Sons, New York, NY, pages 3-11 to 3-38; and Chapter 10 *In: Molecular Cloning: A Laboratory Manual*, 2d Ed., Sambrook *et al.*, eds., pages 10.1 to 10.70). It is also well known in the art to label oligonucleotides and other nucleic acids with nonradioactive labels such as, for example, enzymes, fluorescent moieties and the like (see, for example, Beck, *Methods in Enzymology*, 1992, *216*, 143; and Ruth, Chapter 6 *In*: *Protocols for Oligonucleotide Conjugates (Methods in Molecular Biology, Volume 26)* Agrawal, S., ed., Humana Press, Totowa, NJ, 1994, pages 167-185).

Example 2: Oligonucleotide Targets

[0131] The present invention is drawn to compositions and formulations comprising oligonucleotides or nucleic acids and one or more mucosal penetration enhancers, and methods of using such formulations. In one embodiment, such formulations are used to study the function of one or more genes in an animal other than a human. In a preferred embodiment, oligonucleotides are formulated into a pharmaceutical composition intended for therapeutic delivery to an animal, including a human. Oligonucleotides intended for local or systemic therapeutic delivery, as desired, that may be orally administered according to the compositions and methods of the invention. Such desired oligonucleotides include, but are not limited to, those which modulate the expression of cellular adhesion proteins (e.g., ICAM-1, VCAM-1, ELAM-1), the rate of cellular proliferation (e.g., c-myb, vEGF, c-raf kinase), or have biological or therapeutic activity against miscellaneous disorders (e.g., Alzheimer's, β-thalassemia) and diseases resulting from eukaryotic pathogens (e.g., malaria), retroviruses including HIV and non-retroviral viruses (e.g., Epstein-Barr, CMV).

[0132] Additional oligonucleotides that may be formulated in the compositions of the invention include, for example, ribozymes, aptamers, molecular decoys, External Guide Sequences (EGSs) and peptide nucleic acids (PNAs).

[0133] Various fatty acids, their salts and their derivatives act as penetration enhancers. These include, for example, oleic acid, a.k.a. cis-9-octadecenoic acid (or a pharmaceutically acceptable salt thereof, *e.g.*, sodium oleate or potassium oleate); caprylic acid, a.k.a. n-octanoic acid (caprylate); capric acid, a.k.a. n-decanoic acid (caprate); lauric acid (laurate); acylcarnitines; acylcholines; and mono- and di-glycerides (Lee *et al.*, *Critical Reviews in Therapeutic Drug Carrier Systems*, 1991, page 92). Various natural bile salts, and their synthetic derivatives act as penetration enhancers. The physiological roles of bile include the facilitation of dispersion and absorption of lipids and fat-soluble vitamins (Brunton, Chapter 38 *In: Goodman & Gilman's The Pharmacological Basis of Therapeutics*, 9th Ed., Goodman *et al.*, eds., McGraw-Hill, New York, NY, 1996, pages 934-935). Bile salt derived penetration enhancers include, for example, cholic acid, cholalic acid or 3a,7a,12a-trihydroxy-5b-cholan-24-oic acid (or its pharmaceutically acceptable sodium salt); deoxycholic acid, desoxycholic acid, 5b-cholan-24-oic acid-3a,12a-diol, 7-deoxycholic acid or 3a,12a-dihydroxy-5b-cholan-24-oic acid (sodium deoxycholate); glycocholic acid, (N-[3a,7a,12a-trihydroxy-24-oxocholan-24-yl]glycine or 3a,7a,12a-trihydroxy-5b-cholan-24-oic acid N-[carboxymethyl]amide or sodium glycocholate); glycodeoxycholic acid, (5b-cholan-24-oic acid N-[carboxymethyl]amide-

3a,12a-diol), 3a,12a-dihydroxy-5b-cholan-24-oic acid N-[carboxymethyl]amide, N-[3a,12a-dihydroxy-24-oxocholan-24-yl]glycine or glycodesoxycholic acid (sodium glycodeoxycholate); taurocholic acid, (5b-cholan-24-oic acid N-[2-sulfoethyl]amide-3a,7a,12a-triol), 3a,7a,12a-trihydroxy-5b-cholan-24-oic acid N-[2-sulfoethyl]amide or 2-[(3a,7a,12a-trihydroxy-24-oxo-5b-cholan-24-yl)amino] ethanesulfonic acid (sodium taurocholate); taurodeoxycholic acid, (3a,12a-dihydroxy-5b-cholan-2-oic acid N[2-sulfoethyl]amide or 2-[(3a,12a-dihydroxy-24-oxo-5b-cholan-24-yl)-amino]ethanesulfonic acid, or sodium taurodeoxycholate, or sodium taurodesoxycholate); chenodeoxycholic acid (chenodiol, chenodesoxycholic acid, 5b-cholanic acid-3a,7a-diol, 3a,7a-dihydroxy-5b-cholanic acid, or sodium chenodeoxycholate, or CDCA); ursodeoxycholic acid, (5b-cholan-24-oic acid-3a,7b-diol, 7b-hydroxylithocholic acid or 3a,7b-dihydroxy-5b-cholan-24-oic acid, or UDCA); sodium taurodihydro-fusidate (STDHF); and sodium glycodihydrofusidate (Lee *et al., Critical Reviews in Therapeutic Drug Carrier Systems*, 1991, page 92; Swinyard, Chapter 39 *In: Remington's Pharmaceutical Sciences*, 18th Ed., Gennaro, ed., Mack Publishing Co., Easton, PA, 1990, pages 782-783.

[0134] Unsubstituted and substituted phosphodiester oligonucleotides are alternately synthesized on an automated DNA synthesizer (Applied Biosystems model 380B) using standard phosphoramidite chemistry with oxidation by iodine.

[0135] Phosphorothioates are synthesized as per the phosphodiester oligonucleotides except the standard oxidation bottle was replaced by 0.2 M solution of 3H-1,2-benzodithiole-3-one 1,1-dioxide in acetonitrile for the stepwise thiation of the phosphite linkages. The thiation wait step was increased to 68 sec and was followed by the capping step. After cleavage from the CPG column and deblocking in concentrated ammonium hydroxide at 55°C (18 hr), the oligonucleotides were purified by precipitating twice with 2.5 volumes of ethanol from a 0.5 M NaCl solution.

[0136] Phosphinate oligonucleotides are prepared as described in U.S. Patent 5,508,270, herein incorporated by reference.

Alkyl phosphonate oligonucleotides are prepared as described in U.S. Patent 4,469,863, herein incorporated by reference.

[0137] 3'-Deoxy-3'-methylene phosphonate oligonucleotides are prepared as described in U.S. Patents 5,610,289 or 5,625,050, herein incorporated by reference.

Phosphoramidite oligonucleotides are prepared as described in U.S. Patent, 5,256,775 or U.S. Patent 5,366,878, hereby incorporated by reference.

[0138] Alkylphosphonothioate oligonucleotides are prepared as described in published PCT applications PCT/US94/00902 and PCT/US93/06976 (published as WO 94/17093 and WO 94/02499, respectively).

3'-Deoxy-3'-amino phosphoramidate oligonucleotides are prepared as described in U.S. Patent 5,476,925, herein incorporated by reference.

[0139] Phosphotriester oligonucleotides are prepared as described in U.S. Patent 5,023,243, herein incorporated by reference.

Boranophosphate oligonucleotides are prepared as described in U.S. Patents 5,130,302 and 5,177,198, both herein incorporated by reference.

Methylenemethylimino linked oligonucleosides, also identified as MMI linked oligonucleosides, methylenedimethylhydrazo linked oligonucleosides, also identified as MDH linked oligonucleosides, and methylenecarbonylamino linked oligonucleosides, also identified as amide-3 linked oligonucleosides, and methyleneaminocarbonyl linked oligonucleosides, also identified as amide-4 linked oligonucleosides, as well as mixed backbone compounds having, for instance, alternating MMI and PO or PS linkages are prepared as described in U.S. Patents 5,378,825; 5,386,023; 5,489,677; 5,602,240 and 5,610,289, all of which are herein incorporated by reference.

[0140] Formacetal and thioformacetal linked oligonucleosides are prepared as described in U.S. Patents 5,264,562 and 5,264,564, herein incorporated by reference.

Ethylene oxide linked oligonucleosides are prepared as described in U.S. Patent 5,223,618, herein incorporated by reference.

Peptide nucleic acids (PNAs) are prepared in accordance with any of the various procedures referred to in Peptide Nucleic Acids (PNA): Synthesis, Properties and Potential Applications, *Bioorganic & Medicinal Chemistry*, 1996, *4*, 5. They may also be prepared in accordance with U.S. Patents 5,539,082; 5,700,922, and 5,719,262, herein incorporated by reference.

[0141] With regard to oligonucleotide absorption kinetics, it is important to determine if the oligonucleotide absorption rate function behaves in accordance with diffusion principles or if a zero order (i.e., saturable) process is predominant. This information is useful for formulation design since a zero order process would limit the absolute amount of oligonucleotide absorbed in a given amount

of time. This would direct formulation efforts to release oligonucleotide upstream from the permeable region of the intestine at a rate ≤ k_0 (amount absorbed per unit time) to maximize uptake. However, should the absorption process be independent of concentration, than this formulation approach would not be used since a first-order absorption process would lead to a consistent percentage uptake.

Example 3: Pharmacokinetic studies

[0142] Two studies were designed to address this issue. The firstinvolved the intrajejunal (IJ) administration of increasing doses of ISIS 104838, holding the dose of the penetration enhancer (PE) sodium caprate (C10) constant at 50 mg/kg. The oligonucleotide doses were chosen to bracket the range of oligonucleotide dose considered feasible for human dosage form development- 2.5, 10 and 30 mg/kg. Should saturation occur, the higher doses of oligonucleotide would demonstrate a diminished absolute bioavailability (BAV). The second study tested the hypothesis in a slightly different way by slowly presenting a lower concentration of a high dose of oligonucleotide to an activated segment of intestine (activated by bolus penetration enhancer administration). If a saturable process is present, the slower presentation of oligonucleotide should result in a higher BAV compared to bolus co-administration.

[0143] As can be seen in Table 1 and Figure 1, saturation of oligonucleotide uptake pathways did not occur at the doses studied. The initial responses resulted in higher BAV valuesdue to the inappropriate use of a low dose IV AUC for BAV calculations. After recalculation of the BAVs, it is clear that they are equivalent and therefore the absorption process appears to be linear and not saturable at the doses studied.

Table 1

ISIS 104838 IJ dose (mg/kg)	C10 dose (mg/kg)	Ratio (Oligonucleotide :penetration enhancer)	%BAV (1 mg/kg, IV)	IV dose	%BAV (corrected to relevant IV AUC-extrapolated from other monkey expt.)
2.5	50	1:20	2.1±1.1*	0.125	6.1±3.2
10	50	1:5	3.0±2.2	0.5	5.9±4.4
30	50	1:1.7	10.3±6.1	2	6.8±4.0

average±standard deviation
*AUCs calculated out to terminal time-point-data from sensitive binding plate assay.

[0144] In the second study, oligonucleotide was slowly presented (15 mg/mL @ 30 mg/kg dose) by way of a 30 minute infusion into a region of intestine made permeable by a 50 mg/kg bolus of C10 at t = 5 minutes. As previously postulated, if the absorption process is zero order (i.e., saturable) then this study design would result in increased oligonucleotide uptake over the corresponding (30 mg/kg) oligonucleotide bolus study described above. The resulting comparative bioavailability is presented in Table 2 and the plasma concentrations are shown in Figure 2. There was no significant difference in oligonucleotide BAV. This further supports the conclusion that the oligonucleotide absorption process is first order, at least in the range of oligonucleotide dose-concentrations studied.

Table 2

Animal ID	Bolus	Slow infusion
1	4.2	3.8
2	9.7	10.1
3	11.2	4.4
4	3.5	4.0
5	10.3	4.8
6	1.9	8.3
Average ± SD	1.9	8.3

[0145] The data presented above strongly suggest that the approach for formulation design changes to improve BAV should focus on the sodium caprate penetration enhancer (C10) presentation rather than the oligonucleotide. This is supported by the fact that oligonucleotide is equivalently absorbed by dramatically different presentations (i.e., bolus vs. slow infusion in the face

of equivalent C10 presentation). The rapid absorption and loss of penetration enhancer (PE) from the intestine may limit the area of permeabilization following single bolus PE administration.

[0146] The central idea of this hypothesis is shown in Figures 3A-B, which demonstrates the manner in which current solution dosage forms are believed to present excess PE in the intestinal lumen (Fig. 3A). The excess PE is that amount represented by the area of the PE curve above the so called maximal effect level required for the maximal mucosal permeability enhancement. An example of this occurred during a human intubation experiment when a doubling of the PE level (to 3.3 g) failed to bring about an increased response.

Example 4: Solid dosage formulations for clinical evaluation

[0147] The purpose of this study is to clinically evaluate PEG-based immediate releasing and pulsatile formulations for enhanced oral oligonucleotide absorption by way of rapidly producing and further extending the dynamic action of sodium caprate (C10) by releasing an additional amount of C10 after the initial amount (Fig. 3B). Three types of dosage forms, representing four formulations, were evaluated in humans:

Enteric coated (EC) capsules comprising a single population of immediate releasing (IR) 2 mm minitablets with the full doses of oligonucleotide and C10

EC monolithic tablets comprising the full doses of oligonucleotide and C10

EC pulsed-release capsules comprising both a mixture of IR 2 mm minitablets with the full dose of oligonucleotide and partial dose of C10, and delayed release 2 mm minitablets having the remainder of the C10 dose and lacking oligonucleotide.

[0148] The immediate releasing components of the above two capsule dosage forms (4 formulated batches) are made from, for example, hot-melt granulations of PEG-3350, ISIS 104838 and sodium caprate in a high shear mixer, preferably with a controlled temperature of about 70°C. The granules may be compressed into tablets or minitablets without the use of additional excipients.

[0149] Two approaches are intended for the delayed release (second pulse C10) minitablets. It is believed that a matrixed polymer will have a typical burst release of C10 followed by a sustained release over a designated time. A coated polymer approach is characterized by a lag time with more of a delayed (bolus release) profile rather than that expected from a sustained release (Fig. 1). The delayed release coating approach will be pursued in order to effectively bracket the two parameters

mentioned in dosage form iii) above, that is: the delay time and fractional amount of C10 to be released. Accordingly, the appropriate populations of minitablets are filled into Size 000 capsules and then banded prior to enteric coating with Eudragit L30D-55.

[0150] The pharmaceutical formulations described above may be administered as a single (e.g., 200 mg oligonucleotide in a single tablet) or divided (e.g., 2 x 100 mg oligonucleotide tablets taken at the same time) oral dose once per day in an amount comprising between about 5 mg and 1,000 mg oligonucleotide, preferably between about 100 mg and 500 mg oligonucleotide, and more preferably between about 100 and 200 mg oligonucleotide. Alternatively, the total dosage may be divided and administered as separate dosages two, three or more times per day (i.e., one 100 mg tablet twice per day).

Example 5: In vivo pulsatile study

[0151] Healthy volunteers were dosed with five enteric coated size 000 capsules comprising either immediate release (IR) or pulsatile minitablet formulations.

Granule comprising oligonucleotide, sodium caprate, and PEG 3350 was compressed into 2mm tablets. Similarly, granule composed of sodium caprate and PEG 3350 was compressed into 2mm tablets, and were then coated with a delayed release coating. The appropriate mini-tablets were hand filled into size 000 gelatin capsules, a gelatin band was applied to seal the capsules, and the banded capsules were enteric coated.

[0152] Pulsatile compositions comprised five banded and enteric coated size 000 gelatin capsules comprising immediate releasing 2mm tablets of sodium caprate, the antisense oligonucleotide ISIS 104838, and polyethylene glycol 3350 (first pulse), together with a second population of similar 2mm tablets of sodium caprate, polyethylene glycol 3350, and a delayed release coating (2nd pulse). The delay between pulses is controlled by the amount of delayed release coating on the second population of minitablets. The greater the amount of coating, the longer the delay between pulses. The total dose was 500mg ISIS 104838 and 3.3g sodium caprate (B and C) or 700mg ISIS 104838 with 3.3g sodium caprate (D). See Table 3.

[0153] Immediate release (IR) minitablet compositions (A) consisted of five banded and enteric coated size 000 gelatin capsules containing a single population of 2mm tablets of sodium caprate,

ISIS 104838, and polyethylene glycol 3350. The total dose was 500mg ISIS 104838 and 3.3g sodium caprate.

Table 3 – description of formulations

Test article	Division of sodium caprate between pulses (1^{st}:2^{nd})	Delay between pulses	Dose C10 per capsule (mg)	Dose ISIS 104838 per capsule (mg)
A	NA	NA	660	100
B	50:50	Short	660	100
C	50:50	Long	660	100
D	70:30	Short	660	140

[0154] The pharmacokinetic parameters were compared to those from an earlier composition comprising 10 enteric coated tablets with a total dose of 450mg ISIS 104838, 3.3g sodium caprate, and additional excipients, including polyvinylpyrrolidone, polyplasdone, magnesium stearate, Aerosil, and mannitol.

[0155] Healthy male volunteers were dosed with each formulation after an overnight fast. Phlebotomies were taken at selected time points. Bioavailabilities were determinedby comparison of the area under the curve (plasma concentrations versus time) with IV administration of ISIS 104838 (Table 4). The results are shown graphically in Figure 4. The IR and pulsed release minitablet in capsule formulations resulted in significantly higher bioavailabilities than the large tablets. Among the pulsed release compositions, the short pulse formulation D resulted in the best bioavailability.

Table 4. Bioavailabilities

Test article	avg	stdev	n	Description	Oligo dose
A	4.20	3.36	14	IR	500
B	4.20	2.27	7	Short pulse, C10 50:50	500
C	2.31	1.84	8	Long pulse, C10 50:50	500
D	7.93	5.44	9	Short pulse, C10 70:30	700
E	1.28	1.27	8	Monoliths	450

[0156] Those skilled in the art will appreciate that numerous changes and modifications may be made to the preferred embodiments of the invention and that such changes and modifications may be made without departing from the spirit of the invention. It is therefore intended that the appended claims cover all such equivalent variations as fall within the true spirit and scope of the invention.

[0157] It is intended that each of the patents, applications, printed publications, and other published documents mentioned or referred to in this specification be herein incorporated by reference in their entirety.

What is claimed is:

1. A delayed release oral formulation for enhanced gastrointestinal drug absorption, comprising:

(a) a first population of particles comprising said drug and a penetration enhancer, wherein said drug and said penetration enhancer are released at a first location in the gastrointestinal tract; and

(b) one or more additional populations of particles comprising a penetration enhancer and a delayed release coating or matrix, wherein said penetration enhancer is released in at least one additional location in said gastrointestinal tract downstream from said first location, whereby absorption of said drug is enhanced when said drug reaches said additional locations.

2. The formulation of claim 1, wherein the drug is selected from the group consisting of a protein, peptide, nucleic acid, oligonucleotide, peptide hormone, protease inhibitor, metabolism controlling agent, chemotherapeutic agent, vaccine, monoclonal antibody and polyclonal antibody.

3. The formulation of claim 2, wherein said oligonucleotide is an antisense oligonucleotide.

4. The formulation of claim 2, wherein the penetration enhancer in (a) and (b) is the same.

5. The formulation of claim 2, wherein the penetration enhancer in (a) and (b) is different.

6. The formulation of claim 2, wherein the penetration enhancer is selected from the group consisting of a fatty acid, fatty acid salt, bile acid, bile salt, chelating agent, surfactant and non-chelating non-surfactant.

7. The formulation of claim 6, wherein said fatty acid or fatty acid salt has 8-20 carbon atoms, and linkages between said carbon atoms are either saturated, or one mor more of said linkages are unsaturated, branched or cyclic.

8. The formulation of claim 7, wherein said fatty acid is selected from the group consisting of arachidonic acid, oleic acid, lauric acid, capric acid, caprylic acid, myristic acid, palmitic acid, stearic acid, linoleic acid, linolenic acid, dicaprate, tricaprate, monoolein, dilaurin, glyceryl 1-monocaprate, 1-dodecylazacycloheptan-2-one, an acylcarnitine, an acylcholine, a monoglyceride, or a pharmaceutically acceptable salt thereof.

9. The formulation of claim 6, wherein said bile acid is selected from the group consisting of cholic acid, dehydrocholic acid, deoxycholic acid, glucholic acid, glycholic acid, glycodeoxycholic acid, taurocholic acid, taurodeoxycholic acid, chenodeoxycholic acid, ursodeoxycholic acid, and a pharmaceutically acceptable salt thereof.

10. The formulation of claim 6, wherein said chelating agent is selected from the group consisting of EDTA, EGTA, citric acid, a salicylate, an*N*-acyl derivative of collagen, an *N*-amino acyl derivative of a beta-diketone and a mixture thereof.

11. The formulation of claim 6, wherein said non-chelating non-surfactant is selected from the group consisting of an unsaturated cyclic urea, 1-alkyl-alkanone, 1-alkenylazacycloalkanone, steroid anti-inflammatory agent and mixtures thereof.

12. The formulation of claim 1, wherein said formulation is a capsule, tablet, compression coated tablet, bilayer or trilayer tablet, sachet, liquid-filled capsule or capsule comprising both liquid and solid components.

13. The formulation of claim 1, wherein said particles are bioadhesive.

14. The formulation of claim 1, wherein said particles comprise a substance selected from the group consisting of poly-amino acids, polyimines, polyacrylates, polyalkylacrylates, polyoxethanes, polyalkylcyanoacrylates, cationized gelatins, albumins, starches, acrylates, polyethylene glycol, DEAE-derivatized polyimines, pollulans and celluloses.

15. The formulation of claim 1, wherein said particles comprise a material selected from the group consisting of chitosan, poly-L-lysine, polyhistidine, polyornithine, polyspermines, protamine, polyvinylpyridine, polythiodiethylamino-methylene P(TDAE), polyaminostyrene, poly(methylcyanoacrylate), poly(ethylcyanoacrylate), poly(butylcyanoacrylate), poly(isobutylcyanoacrylate), poly(isohexylcyanoacrylate), DEAE-methacrylate, DEAE-ethyhexylacrylate, DEAE-acrylamide, DEAE-albumin, DEAE-dextran, polymethylacrylate, polyhexylacrylate, poly (D,L-lactic acid), poly (DL-lactic-coglycolic acid) (PLGA) and polyethylene glycol (PEG).

16. The formulation of claim 1, wherein said particles comprise a cationic component.

17. The formulation of claim 16, wherein said particles comprise a complex of poly-L-lysine and alginate, a complex of protamine and alginate, lysine, dilysine, trilysine, calcium, albumin, glucosamine, arginine, galactosamine, nicotinamide, creatine, lysine-ethyl ester and arginine ethyl-ester.

18. The formulation of claim 1 wherein said delayed release coating or matrix is selected from the group consisting of acetate phthalate, propylene glycol, sorbitan monoleate, cellulose acetate phthalate (CAP), cellulose acetate trimellitate, hydroxypropyl methyl cellulose phthalate (HPMCP), methacrylates, chitosan, guar gum, polyethylene glycol (PEG), hydroxypropylmethylcellulose, hydroxypropylethylcellulose, ethylcellulose, hydroxypropylmethylcellulose acetate succinate.

19. The formulation of claim 1, wherein said first population of particles comprises up to about 50% of said penetration enhancer and said additional population(s) of particles comprises the remainder of said penetration enhancer.

20. The formulation of claim 1, wherein said first population of particles comprises at least about 50% of said penetration enhancer and said additional population(s) of particles comprises the remainder of said penetration enhancer.

21. The formulation of claim 1, wherein said first population of particles comprises about 50% of said penetration enhancer and said additional population(s) of particles comprises about 50% of said penetration enhancer.

22. The formulation of claim 21, wherein said first population of particles comprises about 70% of said penetration enhancer and said second population of particles comprises about 30% of said penetration enhancer

23. A method for enhancing the absorption of a drug in a mammal, comprising administering the pharmaceutical formulation of claim 1 to said animal.

24. The method of claim 3 wherein said mammal is a human.

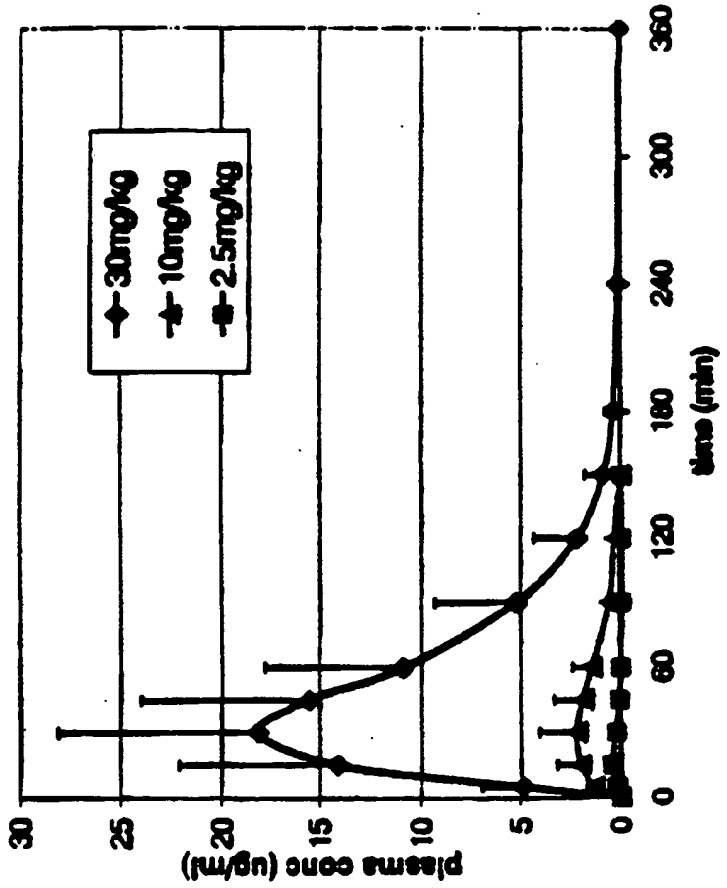

30mg/kg
10mg/kg
2.5mg/kg
plasma conc (ug/ml)
time (min)
0
5
10
15
20
25
30
0
60
120
180
240
300
360

Fig. 1

Fig. 2

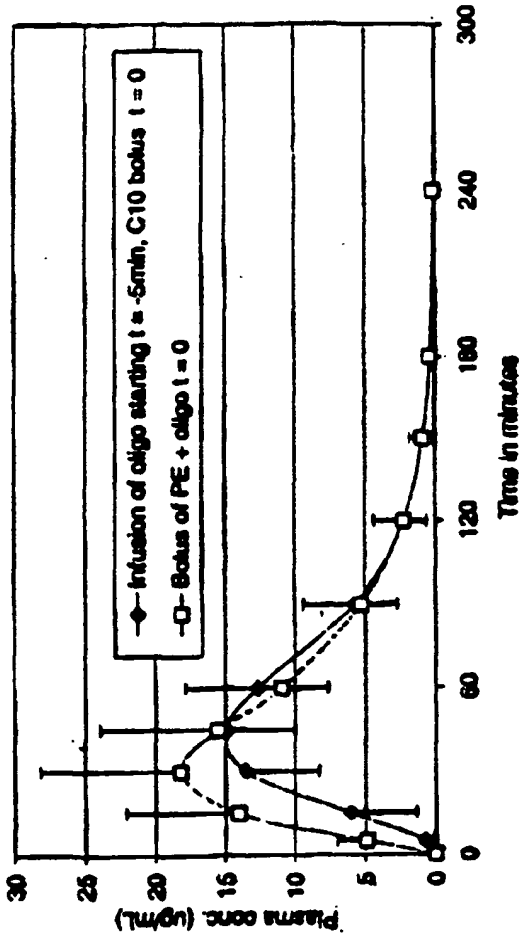

Infusion of oligo starting t = -5min, C10 bolus t = 0
Bolus of PE + oligo t = 0
Plasma conc. (ug/mL)
Time in minutes
0
5
10
15
20
25
30
0
60
120
180
240
300

Fig. 3A

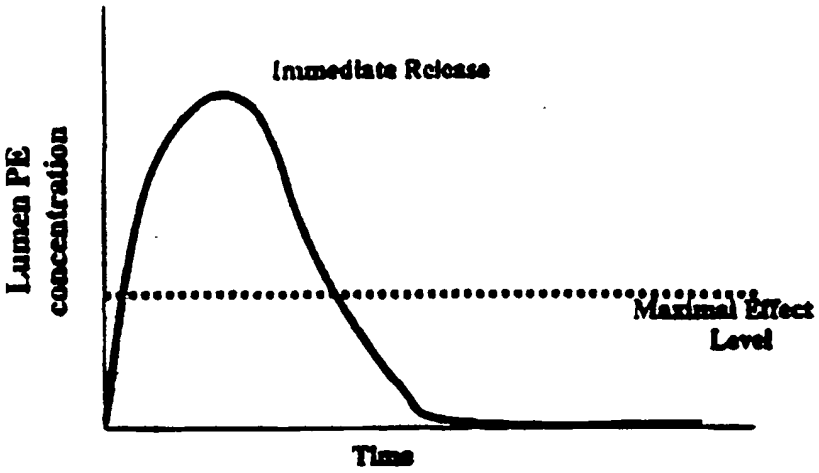
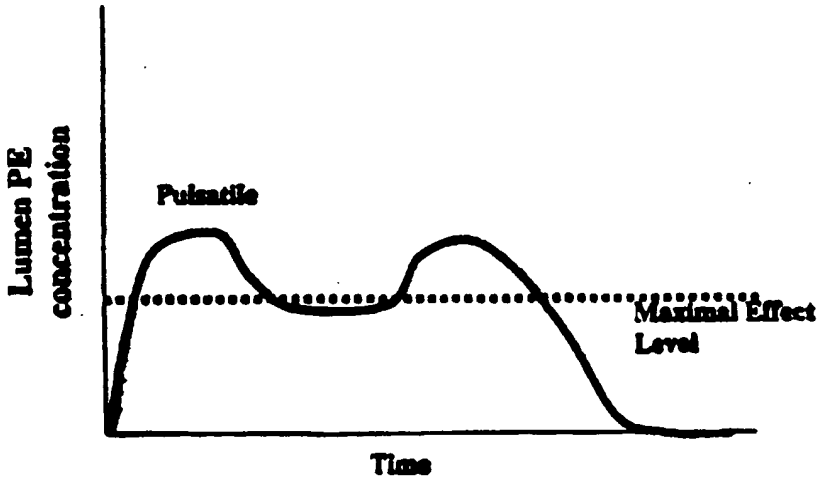

Immediate Release
Lumen PE concentration
Maximal Effect Level
Time

Fig. 3B


Pulsatile
Lumen PE concentration
Maximal Effect Level
Time

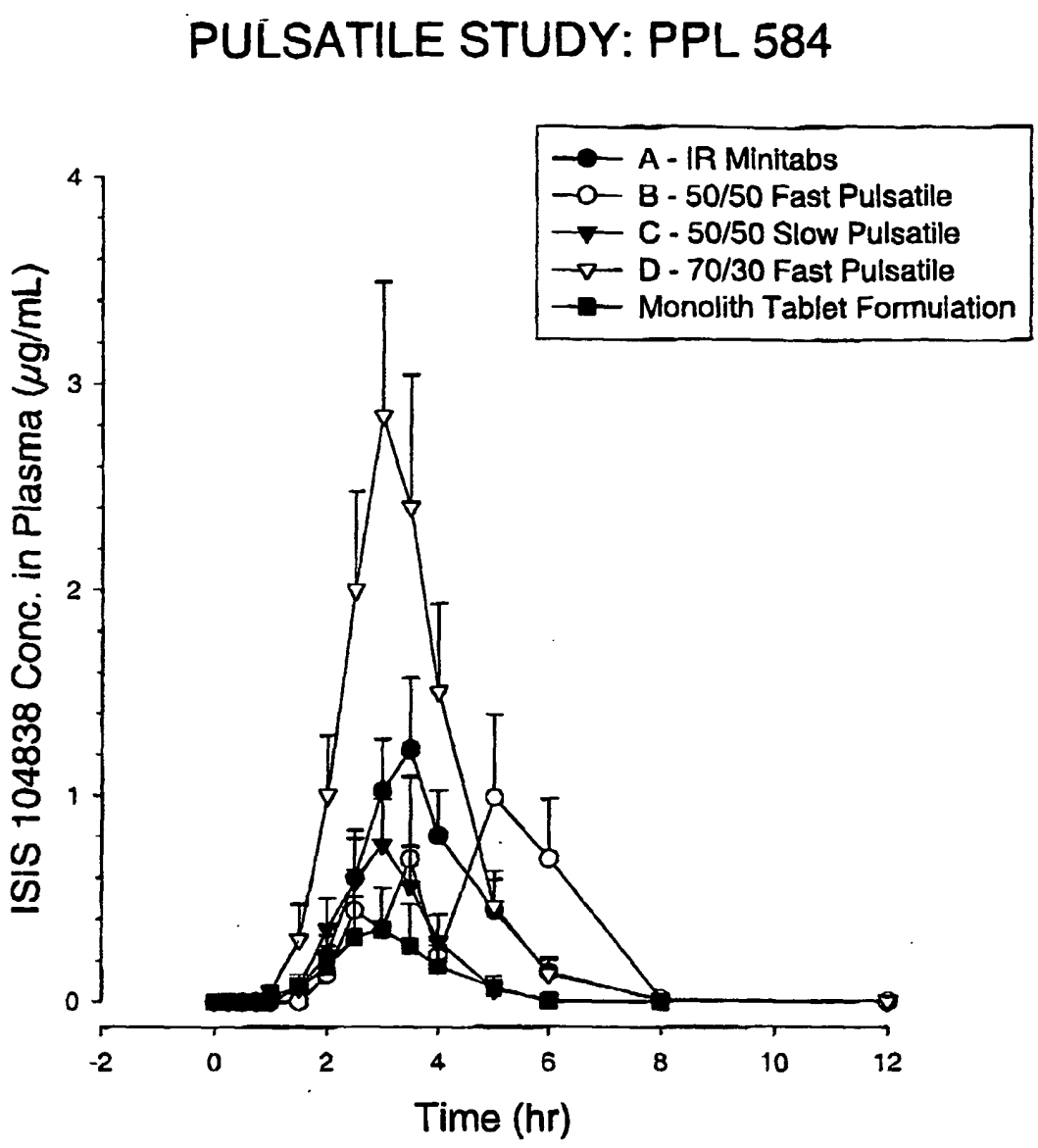

PULSATILE STUDY: PPL 584
A - IR Minitabs
B - 50/50 Fast Pulsatile
C - 50/50 Slow Pulsatile
D - 70/30 Fast Pulsatile
Monolith Tablet Formulation
ISIS 104838 Conc. in Plasma (µg/mL)
Time (hr)
0
1
2
3
4
-2
0
2
4
6
8
10
12

Fig. 4